Physical Factors Influencing Larval Behaviour in Three Species of Solitary Ascidian

Thesis

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PHYSICAL FACTORS INFLUENCING LARVAL BEHAVIOUR IN THREE SPECIES OF SOLITARY ASCIDIAN

by

MARTIN HERBERT DAVIS, B.A.(Hons.), M.Tech.

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Department of Biology
The Open University
Milton Keynes .MK7 6AA
U.K.

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ABSTRACT

Three solitary ascidians, *Ciona intestinalis*, *Ascidiella aspersa* and *Styela clava* coexist in great abundance in Southampton Water, an estuary on the south coast of England. There appears to be zonation of these ascidians on constant-depth submerged substrata (*S. clava* above *A. aspersa* above *C. intestinalis*). As the adults are sedentary, it is suggested that larval zonation in the water column may play an important role in determining the adult distribution. The physical factors gravity, light and hydrostatic pressure are proposed as the cues effecting pre-settlement larval zonation.

Adult ascidians were induced to spawn and monospecific larval populations produced. The responses of these larval populations to light, gravity and hydrostatic pressure, individually and in combination, were determined. The results are used in a qualitative model to identify the depths at which larvae are most likely to congregate; the predictions are compared with the vertical distribution of larvae found in the water column and the distribution of juvenile recruits. Juvenile mortality was eliminated as a cause of adult zonation.

Adult distribution can be explained by pre-settlement larval behaviour. *C. intestinalis* larvae were negatively buoyant, negatively geotactic and ambivalently phototactic; it is suggested that these larvae do not concentrate at a specific depth, but circulate in the water column and settle opportunistically. *A. aspersa* larvae were negatively buoyant, negatively geotactic and positively phototactic; these responses entrain the larvae in a circulation cell below 2 m depth and a buoyancy anomaly concentrates them near the top of the cell, at a depth at which adults are frequently found. *S. clava* larvae were negatively buoyant, negatively geotactic, and positively phototactic (with high barokinesis); these responses concentrate the larvae near the surface, where adults are found in abundance. Interspecific competition is minimised by larval zonation, permitting these three species to coexist.

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Despite all the help provided during the preparation of this thesis, the errors, limitations and omissions remain my own. It is very likely that some of the advice which was offered but ignored, should have been accepted. Next time...

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CHAPTER 1 INTRODUCTION

The present study examines the coexistence of three solitary ascidians in sublittoral fouling communities and attempts an explanation in terms of the physical factors that influence larval behaviour. The questions that will ultimately be addressed are: "Is competition the major organising force in ascidian community structure, or are other biotic or abiotic factors more important?" and "What survival strategy permits these three solitary ascidian species to coexist in such abundance, and what role does larval behaviour play in the strategy?".

1.1 Larval settlement

Clean surfaces submerged in the littoral and sub-littoral marine zones rapidly become colonised by bacteria and diatoms. A succession of marine biota then settle as larvae and spores, which grow to produce a community of fouling organisms. This community usually includes hydroids, barnacles, sea squirts, mussels and, depending on the available light, algae. The initial colonisation process is usually termed fouling, and macrofouling when larger biota settle. It is a dynamic process leading to the formation of a climax community.

Mobile larvae represent the active distribution phase of the life cycle of sedentary fouling animals. To become part of the fouling community these larvae must settle and metamorphose. Before considering community development it is necessary to define some terms. The addition of individuals to a fouling community is termed recruitment. Recruitment is composed of two components, larval settlement and post-settlement juvenile survival; settlement occurs when larvae leave the plankton and take up a benthic existence, but survival is not a discrete process and may change with time. Recruitment is therefore usually defined as the survival of the ensuing individuals until censused by an observer (Keough & Downes, 1982; Keough, 1988).

Recruitment of planktonic larvae is essential for the successful establishment and continued existence of a fouling community.

Larval settlement is the first, and probably the most important, factor determining the structure of a fouling community (Gaines & Roughgarden, 1985). Once the larvae have settled, other factors such as predation and competition can act to shape the climax community. The factors that affect recruitment are often density dependent (Connell, 1985), but the colonisation of a new surface by larvae depends only on the surface being suitable and present in the right place and at the right time. This does not mean that the choice of a settlement site is random or unimportant; in fact, this choice by the planktonic larva is critical to the subsequent survival of the adult since the location of the settlement site largely determines the environmental conditions experienced by the adult. The statement merely emphasises that the two most important variables affecting settlement potential are temporal and spatial variation of larval density.

Many factors influence the settlement of the larvae. Larval behaviour is one factor thought to have a major effect on the spatial distribution of planktonic larvae and on settlement (Meadows & Campbell, 1972). However, the intensity of settlement at a suitable site is initially dependent on the availability of larvae to settle. This is the temporal component; unless the larvae are present in the water column, the substrate will not be colonised by that species. Indeed, large scale temporal variations in settlement have been attributed to seasonal differences in numbers of larvae produced (Sutherland & Karlson,1977; Yoshioka, 1986; Gotelli,1987), which are often correlated with temperature or photoperiod (Giese & Pearse, 1974).

Patterns of larval settlement depend upon a spatial component. This component has two aspects; the proximity of the substrate to the breeding adults, and the position or orientation of the

substrate in the water column. The importance of the former factor is a function of the length of time spent in the planktonic phase, and that of the latter factor reflects the behaviour of the larvae and their ability to select settlement sites. For example, spatial distributions of larvae in the plankton have been shown to influence the location of settlement (Cameron & Rumrill, 1982; Grosberg, 1982; Yoshioka, 1982; Gaines *et al.*, 1985; Olson,1985) and settling larvae have been shown to respond to a variety of substrate characteristics, such as composition and orientation, in addition to siting (Dybern, 1963; Young & Braithwaite, 1980). For organisms with short-lived larvae, such as colonial ascidians, time of release closely corresponds to settlement time. Most colonial ascidians examined have been observed to release larvae only during the day (Olson, 1983; Svane & Young, 1989). For these larvae, behavioural response to light may be an important factor in selecting suitable locations for settlement.

The fouling communities that developed on the dock walls at Fawley (on the south coast of England) experience varying depths of submersion. They exhibit zonation, which may be achieved by at least two mechanisms. In the first, common to many fouling organisms e.g. barnacles, larvae settle on substrate throughout the water column but may be reduced to a discrete zone as a result of competition (Connell, 1961). In the second mechanism the larvae select a suitable site for optimum adult survival.

The top of the ascidian zone on the dock wall is just above the spring tide low water level. Only species capable of withstanding some degree of desiccation and exposure to ultra-violet radiation (Jokiel, 1980), such as the immigrant *Styela clava* Herdman, are found at this level. Further down the wall, in the permanently submerged zone, *S. clava* is replaced by *Ascidiella aspersa* (Müller) and below this is a zone consisting mainly of *Ciona intestinalis* (Linnaeus). This zonation could be explained by competition. However, an examination of recently laid mooring

chains (less than three years old) in the area indicated that, despite being removed from the selective pressure of occasional exposure, the three ascidian species were frequently zoned on the chains with increasing depth in the same order as on the dock wall, i.e. *S. clava*, then *A. aspersa* and then *C. intestinalis*. In general *S. clava* is found near the surface; for example, the vertical edges of a floating pontoon at Fawley was fouled almost exclusively with *S. clava* from a depth of 5 cm, whereas *A. aspersa* exhibited an initial intense recruitment on ropes at a depth of between 1.5 and 2.5 m. *C. intestinalis* did not exhibit such clear zonation, the underside of some buoys floating on the surface and other horizontal surfaces at a variety of depths were found to support small populations of this ascidian; it was often found with *A. aspersa* on mooring ropes and occasional populations were found above the *A. aspersa* zone. On substrates exposed for more than five years, ascidian recruitment occurred on suitable substrata throughout the water column, blurring the initial zonation.

These fortuitous observations suggested that the larvae of some ascidian species could, to some degree, control the depth at which they settle and, therefore, their depth in the water column. However, Dalby & Young (1992) dismissed larval zonation as an explanation for the similar recruitment zonation of *Styela plicata* on fouling panels, attributing it instead to early post-settlement mortality. Ascidians settle as tadpole larvae whose behaviour appears to be variable, but most are capable of responding to certain stimuli such as light and gravity (Berrill, 1975; Cloney & Tottence, 1983; Svane & Young, 1989). The larvae of the three most abundant species of solitary ascidian in the Fawley inlet channel all possess very basic sensory receptors, which are unlikely to be capable of detecting gradations in these stimuli. Zonation of ascidians on constant-depth (floating) substrata, if it is a genuine phenomenon, generates two crucial questions: how do the larvae control their depth prior to settlement, and what is the ecological significance of such sub-littoral zonation?

1.2 Potential cues for depth control in the water column

The physical stimuli that could provide cues for larval depth control are temperature, dissolved oxygen, salinity, light, gravity and hydrostatic or bathymetric pressure. Stimuli can elicit two types of response: change in orientation or change in the level of locomotor activity. A directional response to a stimulus is termed a taxis, and requires a discernible source of the stimulus towards or away from which the organism can move. Movement towards the stimulus is termed positive taxis and movement away is termed negative taxis. Light (phototaxis) and gravity (geotaxis) are the principle orientating stimuli in water that can generate a directional response in the vertical plane (Fraenkel & Gunn, 1961).

Temperature, dissolved oxygen, salinity, pressure and light quality are all stimuli with no discernible source. Variations in these stimuli can only be experienced by moving to a new position. Thus these stimuli are more likely to stimulate motion rather than elicit an orientation response. Changes in the level of locomotor activity in response to external stimuli are termed 'kinesis' responses (Fraenkel & Gunn, 1961). High kinesis results from increased activity with an increase in stimulus intensity; low kinesis, from an increased activity in response to a decrease in stimulus intensity. Kinesis reactions occur without regard to stimulus direction. It should be noted that light can act as a kinesis stimulus (light quality) and a taxis stimulus (light source). The kinesis stimuli most relevant to the present study are likely to be temperature, dissolved oxygen, salinity, light (quality) and hydrostatic pressure. In the marine environment hydrostatic pressure is the most conservative of these stimuli being ubiquitous and varying predictably with depth.

Biological cues, such as the presence of con-specifics, will only operate over short distances and are therefore unlikely to be involved in pre-settlement behaviour.

Water temperature varies with depth but the variation encountered in an estuary is not readily predictable, depending on the area of shallow water or mud flat and the degree of mixing that occurs, which in turn depends upon factors such as the weather and the topography of the estuary floor. Similarly, although in general the concentration of dissolved oxygen will be greater in the surface layers of the estuary water, it is unlikely that any consistent gradient of dissolved oxygen concentration with depth will exist. Therefore these two potential cues can be excluded because of the variable nature of the mixing processes in an estuary. Salinity will vary within an estuary depending upon the state of tide. Although the variation is reasonably predictable, it is likely to be small at the seaward end and will also be subject to estuarine mixing processes, rendering it an inappropriate cue for depth control.

1.2.1 Light

Light has been proposed as the principle ecological factor controlling the vertical distribution of planktonic animals (Thorson, 1964), but it is a highly variable parameter in the estuarine environment. To be of use as a single cue for position fixing in the water column it would need to be constant at source yet predictably attenuated with depth. However, the intensity and quality of light observed at a specific depth are dependent upon the amount and type of dissolved and suspended matter present in the water, the time of day, the season, and local weather conditions. The consequent variability in light penetration renders this stimulus useful only as a directional cue. Nevertheless, the majority of previous studies have examined the effect of light on the settlement of ascidian larvae.

Early workers reported that the larvae of many ascidian species responded to light (Grave, 1920; Mast, 1921, Grave & Woodbridge, 1924, Grave, 1935) and that the tadpole larvae of almost all

ascidians are induced to swim by a sudden decrease in light intensity - the shadow response. Those larvae with well-developed light responses possess a single asymmetrically placed eye with pigment cup and lens. They apparently exhibit an initial photopositive phase just after liberation but are photonegative at the time of settlement; examples are given in Table 1.

TABLE 1 **Ascidian larvae with well-developed light responses**

Species	Reference
Amaroucium constellatum (Verrill)	Grave, 1920; Mast, 1921.
Amaroucium pellucidum (Leidy).	Grave, 1920; Mast, 1921.
Ascidia callosa	Young, 1982.
Ascidia nigra	Grave, 1935; Goodbody, 1963.
Ascidia paratropa	Young, 1982.
Ascidiella aspersa	Holmes, 1968.
Boltenia villosa	Young, 1982.
Botryllus schlosseri (Pallas)	Grave & Woodbridge, 1924; Woodbridge, 1924.
Ciona intestinalis (Linnaeus)	Berrill, 1947; Millar, 1953; Dybern, 1963.
Corella inflata	Young, 1982.
Diplosoma listerianum	Crisp & Ghobashy, 1971.
Polyandrocarpa gravei	Grave, 1935.
Polyandrocarpa tincta	Grave, 1935.
Pyura haustor	Young, 1982.
Styela clava	Holmes, 1968
Styela gibbsii	Young, 1982.
Symplegma viride	Grave, 1935.
Trididemnum solidum	van Duyl *et al.*, 1981.

It should be noted that the majority of these observations were carried out in shallow water (dishes, 20 cm tubes etc.). To respond to any environmental cue, the larvae must be active and

mobile; if larval activity is initiated by pressure or by movement of water past surface cells as, for example, when larvae are sinking (c.f. insect flight response to air movement), then it is possible that there will have been insufficient depth for the activity response to be triggered and any cycle of passive sinking followed by active swim-up would be interrupted at the passive sinking stage, and could therefore be interpreted as negative phototaxis.

Other ascidian larvae appear to be indifferent to light but may swim upwards in response to gravity. Examples are given in Table 2.

TABLE 2 Ascidian larvae that appear to be indifferent to light

Species	Reference
Dendrodoa grossularia	Berrill, 1950.
Distaplia sp.	Berrill, 1948.
Molgula citrina	Grave, 1926.

Some ascidian larvae overlap both of these categories; the eye of *Cynthia* (= *Styela*) *partita* is poorly developed and the directional responses of the larvae to light are weakly developed in comparison with the response to gravity (Grave, 1941; 1944). Others fit neither category; the larvae of *Chelyosoma productum* do not exhibit any predictable behaviour with regard to light or gravity (Young & Braithwaite, 1980) and the larvae of *Ascidia mentula* appear to be negatively phototactic irrespective of age (Svane, 1987). Thus it would appear that the response of ascidian larvae to the presence of light is species specific.

The majority of ascidian larvae also respond to sudden absence of light; they show a strong tendency to swim upwards in response to shading (Grave, 1920; Mast, 1921; Woodbridge, 1924). Even *Cynthia partita* larvae, with poorly developed eyes, respond vigorously to shade (Grave, 1944), suggesting that light (presence or absence) acts as a strong directional cue.

Woodbridge (1924) suggested that this shadow response enabled the tadpole larvae of *Botryllus schlosseri* to find the underside of eelgrass blades, which appear to be suitable sites for attachment and growth. It was presumed that larvae drifting through the shadow cast by the blades would be stimulated to swim upward thus contacting the plants. She demonstrated experimentally that more larvae settled on an eelgrass blade suspended diagonally across a culture dish than on the bottom or sides of the dish, but the experiments were carried out under artificial light conditions (unspecified) and were not controlled for differences in substratum texture and composition, now thought to be important cues used by larvae selecting habitats for settlement (Young & Braithwaite, 1980; Schmidt, 1982; Young, 1982).

A necessary prerequisite to estimation of the significance of larval responses to environmental cues is a consideration of what cues are likely to be encountered by the larvae. The diel release or hatching time of larvae can determine the environmental conditions available to larvae as settling cues (e.g. light, temperature, tidal height) which, in turn, can influence the location of settlement. Many organisms show diel patterns in larval release. Light is known to trigger larval release in colonial ascidians (Millar, 1971), suggesting that larvae are released in the field shortly after dawn. Diel release time can influence initial survival of larvae due to differences in predator activity (Hobson & Chess, 1978); visual predators may be avoided by night time release of larvae, as accomplished by some corals (Richmond & Jokiel, 1984).

For organisms with a short larval phase, such as colonial ascidians, the time of release must closely correspond to settlement time, and initial behaviour patterns will be crucial for the selection of suitable settlement sites. Most colonial ascidians examined have been observed to release larvae only during the day (Olson, 1983; Svane & Young, 1989). For these larvae, light may be an important factor in selecting suitable locations for settlement. However, for larvae that

spend longer in the water column, the behavioural patterns that influence the location of settlement sites may be different from the initial behaviour; furthermore, the behaviour involved in the selection of settlement sites may itself be influenced by the diel timing of settlement because some environmental cues available to settling larvae, such as light intensity, vary with time of day. Larvae of many species are responsive to light level at the time of settlement in both the laboratory (Crisp & Ghobashy, 1971; Meadows & Cambell, 1972; Miller & Hadfield, 1986) and the field (Olson, 1983). Determination of the diel time of larval hatching may indicate the importance of light as a cue in larval behaviour prior to settlement.

It has been suggested that the juveniles of most sublittoral ascidians require cryptic sites for survival (Dybern, 1963; Goodbody, 1963; Young & Chia, 1984). Negative phototaxis is usually suggested as the mechanism whereby larvae select such sites (Berrill, 1950; Crisp & Ghobashy, 1971; Young, 1982) and the shadow response as a mechanism to increase the chance of a larva finding a shaded site. Dybern (1963) divided small aquaria into a variety of light and dark sections to show that the larvae of *C. intestinalis* preferentially select the dark sections as the most suitable site for attachment, but his artificial light source did not readily translate to the natural environment. He suggested that the shadow response proposed by Woodbridge (1924) could be applied to the behaviour of the larvae of *C. intestinalis* since he observed that "a great many" of the larvae in his experiments had settled within the dark painted zones of the aquaria. However, it is difficult to reconcile this suggestion with his observation that approximately 75% of all the *C. intestinalis* specimens in the region that he had studied had occurred on steeply sloping rock walls, on horizontal surfaces and in caves, all at depths of up to 10 m, rather than on the underside of overhanging rock ledges nearer the surface.

Young & Chia (1985) tested ascidian tadpole shadow response for eight species in half-darkened petri dishes exposed to light. They found that in both continuous light and alternating light/dark treatments, larvae of most species located dark regions more often than could be expected by chance alone. Nevertheless they concluded that their data did not support the hypothesis that the ascidian shadow response helps larvae locate shaded or overhanging settlement sites.

1.2.2 Gravity

Gravity has also been proposed as a cue for vertical movement of larvae in the water column. It is ubiquitous and a more stable environmental parameter than light. However, I believe it should be viewed only as a directional cue for the present study, since the variation in the gravitational force over the three or four metres depth encountered by larvae in the Fawley Intake channel would be so small as to render it an unreliable cue for depth control, unless the larvae possess a hitherto undetected ability to discriminate minute changes in gravitational force. Thus gravity alone is unlikely to be a stimulus for depth control of ascidian larvae. Nevertheless, several studies have examined the effect of gravity, with and without light, on ascidian larvae.

Crisp and Ghobashy (1971) studied the responses of *Diplosoma listerianum* larvae and found that they were influenced mainly by a strong negative response to gravity. This study is unusual in that a variety of light intensities was applied, and measured, to test both phototactic and photokinetic behaviour. Light experiments were carried out in the horizontal plane in order to eliminate the effect of gravity; the results indicated that in the absence of any gravitational influence the larvae were positively phototactic until just before metamorphosis when they exhibit a reversal in phototaxis. The authors observed no significant photokinetic behaviour. The response to gravity was determined by intermittent observation of larvae in a vertical tube

(20 cm), maintained in the dark apart from observation periods; the response proved to be temperature dependent. The response of larvae to the combined stimuli of light and gravity was tested in the vertical tube with light reinforcing and opposing gravity. They reported that just before metamorphosis the larvae became negatively phototactic as well as negatively geotactic.

Svane (1987) found that the larvae of *Ascidia mentula*, kept in tubes, distributed themselves mainly towards the bottom of the tubes irrespective of light conditions. The larvae were shown to be negatively phototactic but gravity was not thought to be an important stimulus for this species; however, this was only tested by changing the direction of the light stimulus and no attempt was made to attenuate the effect of gravity. I consider that this study and that of Crisp and Ghobashy (1971) use a too simplistic approach for determining the response to gravity. Merely changing the vertical direction of the light source ignores any difference in magnitude of the responses to light and gravity. Consider, for example, larvae that show strongly negative geotaxis and weakly positive phototaxis. These will swim up in the water column when light is applied from above and, as the response to gravity is stronger than the response to light, they will still swim up if light is applied from below, albeit with a less intense response. A cursory examination of the results of these experiments would suggest that the larvae are unaffected by light. Therefore great care in experimental design is needed when attempting to determine the response to environmental cues, in case the observed distribution is due to the balance of several responses.

Young (1982) studied the settlement responses of the larvae of twelve species of solitary ascidians to light, gravity and abiotic surface features (e.g. cracks). He observed the settlement of larvae in half-darkened petri dishes submerged in shallow sea-water and maintained below a light bulb. He determined the visual threshold of the larvae and used their response to monochromatic

light to determine the wavelength of light to which they were most sensitive. However his settlement experiments were carried out with an artificial light source, sometimes enhanced by natural daylight, providing a continuous but variable level of illumination, and the larval response to gravity appears to be based only on observation of tadpole swimming and sinking behaviour.

1.2.3 Hydrostatic pressure

The pre-eminent cue for the control of larval depth in the water column would appear to be hydrostatic, or bathymetric, pressure. Response to small changes in hydrostatic pressure has been reported for a wide range of planktonic organisms and has been postulated as a depth regulatory device (Knight-Jones & Morgan, 1966). However, pressure does not provide a directional cue (Digby, 1967). If there is an optimal hydrostatic pressure, and thus depth, for larval settlement, the larvae can only find it by trial and error. Rice (1964) found that the first stage larvae of twenty-two species of decapods reacted to pressure increases by increasing locomotor activity and to pressure decreases by decreasing such activity, but direction of movement was not a function of pressure. Crisp & Ghobashy (1971) appear to be the only workers to have studied the effect of pressure on the settlement of ascidian larvae. They found that hydrostatic pressure in the range 0.5-2.0 atmospheres did not effect the settlement behaviour of *Diplosoma listerianum* and concluded that there was no detectable depth regulation mediated by pressure; but they carried out these experiments within a narrow temperature range (18-20°C) despite having demonstrated a reversal of larval response to light and gravity between 12°C and 19°C.

A review of the literature indicates considerable variability in the settlement behaviour of ascidian larvae and suggests that the effect of hydrostatic pressure on settlement has been neglected. Initially it would appear that no single cue is capable of providing the stimulus necessary for

ascidian larvae to maintain station in the water column. Although each stimulus will have to tested in isolation, it will also be necessary to examine the interaction between stimuli to determine whether the balance of cues can generate the necessary behaviour to regulate depth.

For the species of interest in this study, the effects of light and gravity on larval settlement have only been studied in depth for *C. intestinalis* (e.g. Millar, 1953; Dybern, 1963); few studies have been carried out on the larvae of *A. aspersa* (Holmes, 1968) and *S. clava* (Holmes, 1968). Many of the experiments described in the literature are confounded by the lack of control of critical factors such as temperature, light intensity and quality, and much work appears to have been directed only at explaining the shadow response. It is always difficult to translate laboratory results to the field situation, but I cannot reconcile the reported larval responses with my observations of initial recruitment on vertical bands of substratum in the sub-littoral zone where the only shadow sources is the buoy supporting the substratum. There is also a continuous unidirectional current to draw away any larvae responding to a recent shadow before they can settle. Recruitment of at least one species of solitary ascidian occurs within a narrow well-defined band at constant depth, so it is unlikely that these larvae are responding to previously encountered shadows, and possible that they are capable of maintaining station in the water column prior to settlement. This phenomenon needs to be examined in more detail.

1.3 The objectives

Many chemical and physical cues are thought to influence the settlement of marine invertebrates, such as the neurotransmitter GABA (Qian & Bryan, 1996), surface texture (Hills & Thomason, 1996), presence of microbial biofilms (Tsurumi *et al.*, 1996; Satuito *et al.*, 1996). These cues operate over a relatively short range and can initiate the adhesion stage of settlement. The

present study is concerned with events prior to settlement, and the cues that bring larvae into the neighbourhood of suitable settlement sites. The hypothesis to be tested is that hydrostatic pressure is a critical factor in the pre-settlement zonation of the larvae of *S. clava*, *A. aspera* and *C. intestinalis*. I will not, therefore, determine the effects of light, gravity or pressure on settlement, but rather on the position of the larvae in the water column prior to settlement. To do this I will examine larval phototaxis and geotaxis in the laboratory, monitor larval distribution in the field and attempted to reconcile these observations with the recruitment patterns observed locally. Although some of the experiments previously identified in the literature may warrant repetition under more controlled conditions, I felt that a different approach using novel techniques will be required to test the importance of hydrostatic pressure in larval behaviour.

If the hypothesis is supported, it may be possible to model the pre-settlement site selection process. A model for the depth regulation of zooplankters has been proposed by Schöne (1975) in which the vertical position of the organisms is the net result of complex interactions among morphological and behavioral traits. The model starts with a consideration of the buoyancy of the organism. The effect of buoyancy can then be modified by locomotor responses mediated by the behavioral repertoire of the species to the principle orientating cues. Thus positively buoyant larvae that exhibited positive geotaxis would have to swim actively away from the surface, the swimming response activated by light or by the reduced hydrostatic pressure. Cessation of swimming would result in the larvae rising. Therefore depth could be controlled by the balance between swimming activity and buoyancy. Similarly, negatively buoyant larvae would have to swim to rise to the surface or even to maintain station in the water column. This response could be triggered by reduced light level or, more likely, increased hydrostatic pressure. A similar approach has been used by Sulkin (1984) to explain the depth regulation exercised by the larvae of brachyuran crabs. However, I believe that a qualitative model must start at an earlier stage if it

is to fully explain larval behaviour; it must commence at the starting point of larval behaviour, i.e. hatching. Therefore an estimate of the depth at which the eggs float prior to hatching is essential. Modulation of larval buoyancy by, for example, phototaxis, geotaxis or barokinesis must act from this point in the water column.

1.4 Ecological significance of larval depth control

If some ascidian larvae are capable of maintaining station in the water column and selecting a settlement depth, then the final question to be addressed is "what is the ecological significance of this phenomenon?" As larval settlement determines the ecological niche available to be exploited by the adult, it is possible that the larval behaviour of these three species influences adult survival, possibly maximizing it by reducing competition and promoting coexistence.

Two or more species that exist in the same habitat and utilise the same limited resource(s) will be in competition, which will either be of the scramble type, where each individual gets a little of the resource(s) but may well die, or a contest type where only some, competitively superior, individuals will get enough of the resource(s) to survive (Nicholson, 1954). The degree of competition experienced by the species is proportional to the similarity of their fundamental niches. The maximum actual competition occurring in any particular situation is determined by the similarity of the realised niches in that situation, and the proximity of the populations to the carrying capacity of the environment (Begon *et al.*, 1990). Competition between solitary ascidian species is probably most severe during the larval settlement season, as it is in most sessile species, because settlement space is usually the limiting resource; food supply is unlikely to present as great a problem. Indeed, Holmes (1968) considered that competition for food in the adult stage is probably non-existent. Of

special interest is the relationship of the larvae of any introduced species, such as *S. clava*, to those of the indigenous forms with which it might come into competition, particularly pre-settlement behaviour when competition between them would be most marked.

Excluding competition with native forms, the greatest factors affecting the ecological success of an introduced species such as *S. clava* will involve the relationship of the animal with the physical environment. A species can only successfully colonise an area outside its previous range when the physical factors of the environment, mainly temperature, allow it to breed successfully. It would appear that the temperature regimes of southern English waters are similar to those of its original range (Wallace, 1961) and are suitable for *S. clava* to breed successfully (Holmes, 1968).

The competitive exclusion principle (Gause, 1934; Hardin, 1960) states that in a stable environment, two species can only coexist if a niche difference occurs between them. If no difference exists then the superior competitor will eliminate the inferior one from that habitat. There has been much discussion on the relevance of this theory to ecology (see, for example, Strong *et al.*, 1984; Shorrocks *et al.*, 1984; den Boer, 1986). The questions that must be considered in the present study are: "Is competition the major organising force in ascidian community structure?" and "What survival strategy permits these three solitary ascidian species to coexist in such abundance?".

Competition can be symmetric or asymmetric. In intraspecific competition the competitors share the same fundamental niche and the competition can range from symmetrical to highly asymmetrical, depending on the individual's status. For example, two identically sized, clonal animals are likely to have very similar competitive abilities, whereas two individuals

of differing size and developmental state, such as siblings which hatched or settled at different times, are likely to have very different competitive abilities. However, interspecific competition is usually asymmetrical, with one species being affected to a much greater extent than the other (see, for example, Jackson, 1979; Lawton and Hassell, 1981; Grace and Wetzel, 1981). Interspecific competition is most relevant to the present study.

Mechanisms of survival fall into two broad categories. To survive in the short term animals must, of course, be adapted to the particular environments in which they live. However, other properties of animal populations can be regarded as "adaptations to the pattern of the environment in space and time" (Levins, 1968); these properties involve flexibility of response to environmental factors, and it is these adaptations which are characterised as strategies. Tactics are mechanisms employed to attain a particular, defined goal which, for living reproducing organisms, is the perpetuation of their genes in future generations. Tactics which maximise numbers of surviving offspring are self-perpetuating through genetic inheritance and develop into strategies. MacArthur (1955) suggests that efficiency and stability are the two features necessary for survival under natural selection; I hope to be able to identify certain aspects of the behaviour of the larvae of solitary ascidians which ensure the fulfilment of these two criteria in adult populations and communities.

There have been many strategies suggested for the avoidance of competition. For example, feeding strategies can reduce potential competition with other filter feeders. The ascidian food gathering apparatus is relatively large in relation to the size of food particles (Jørgensen and Goldberg,, 1953; Jørgensen, 1955; Holmes, 1968), so they have the ability to feed on a wide range of food particle sizes in a relatively unspecialised manner. This can be regarded as just one aspect of the relationship between environmental grain, in the sense of MacArthur and Levins

(1964), and the size of the organism living in that environment. MacArthur (1955) has shown that a given stability can either be achieved by a large number of species with specialised diets or by a smaller number of species with catholic diets - the latter appears to be the case for ascidians in Southampton Water. Indeed, the fauna of estuaries is generally characterised by having relatively few species, but these few species may be extremely abundant. MacArthur (1955) states that "natural selection operates for maximum efficiency subject to a certain necessary stability". It is possible that ascidian species may achieve efficiency by virtue of their wide diet and, because wide diet increases stability, this is compensated for by a low species diversity.

Niche differentiation is an example of an avoidance strategy permitting coexistence. This can occur either by chance, as the result of current competition (Begon *et al.*, 1990) or as the "ghost of competition past" (Connell, 1980). Enemy free space (Jefferies and Lawton, 1985) is another mechanism for coexistence; in this strategy there is a 'safe' portion of the habitat which is unavailable to the superior competitor and allows the inferior competitor to survive. In respect to this strategy, it should be noted that the superiority of a competitor may vary depending on the environment under which the competition occurs, allowing a competitor which is inferior in some environments to be superior in others (Tansley, 1917; Park, 1954; Connell, 1961).

Reproductive strategies optimise the number of offspring that can efficiently utilise available resources. Spawning at different times of the year may reduce competition for settlement sites and maximise the opportunities for recruitment of individuals of a species. Extending the life cycle to longer than a year may enable individuals to survive as actively feeding adults during periods of shortage sufficient to kill juveniles, so that they can reproduce again in more suitable environmental conditions during the next breeding season.

Coexistence can occur in patchy, heterogeneous habitats by a process of local extinctions and re-invasions (Brown, 1989; Hastings, 1990). Habitat instability (Hutchinson, 1961), parasitism, disease and predation (Begon *et al.*, 1990) can all foster coexistence by maintaining a population at a level below that at which limitation through competitive interactions would occur. It is unfortunate that so little work appears to have been done on *S. clava* in its original range, the north-western Pacific, which could help to identify the factors or adaptations which have allowed this species to coexist and thrive in its new, European environment. Nevertheless, I hope to identify some of the strategies which have aided the penetration and colonisation of south coast estuaries by *S. clava*.

Finally, it should be stressed that successful recruitment of planktonic larvae is crucial to the establishment and maintenance of populations of sessile marine invertebrates. It has been shown that patterns of larval settlement can be the main determinant of community structure (Underwood and Denley, 1984; Connell, 1985; Gaines and Roughgarden, 1985; Keough, 1988). Larval supply can influence location and intensity of settlement, and variations in the timing and location of settlement can affect both larval and juvenile survival and hence recruitment rates (Keough and Downes, 1982; Gaines and Roughgarden, 1985). However, the settlement location itself may be crucial to subsequent survival (Keough and Downes, 1982) so pre-settlement larval behaviour is a critical step in the recruitment process. Identifying the factors that influence the pre-settlement behaviour of larvae can thus contribute to an understanding of the mechanisms that determine community structure.

CHAPTER 2 THE STUDY SITE

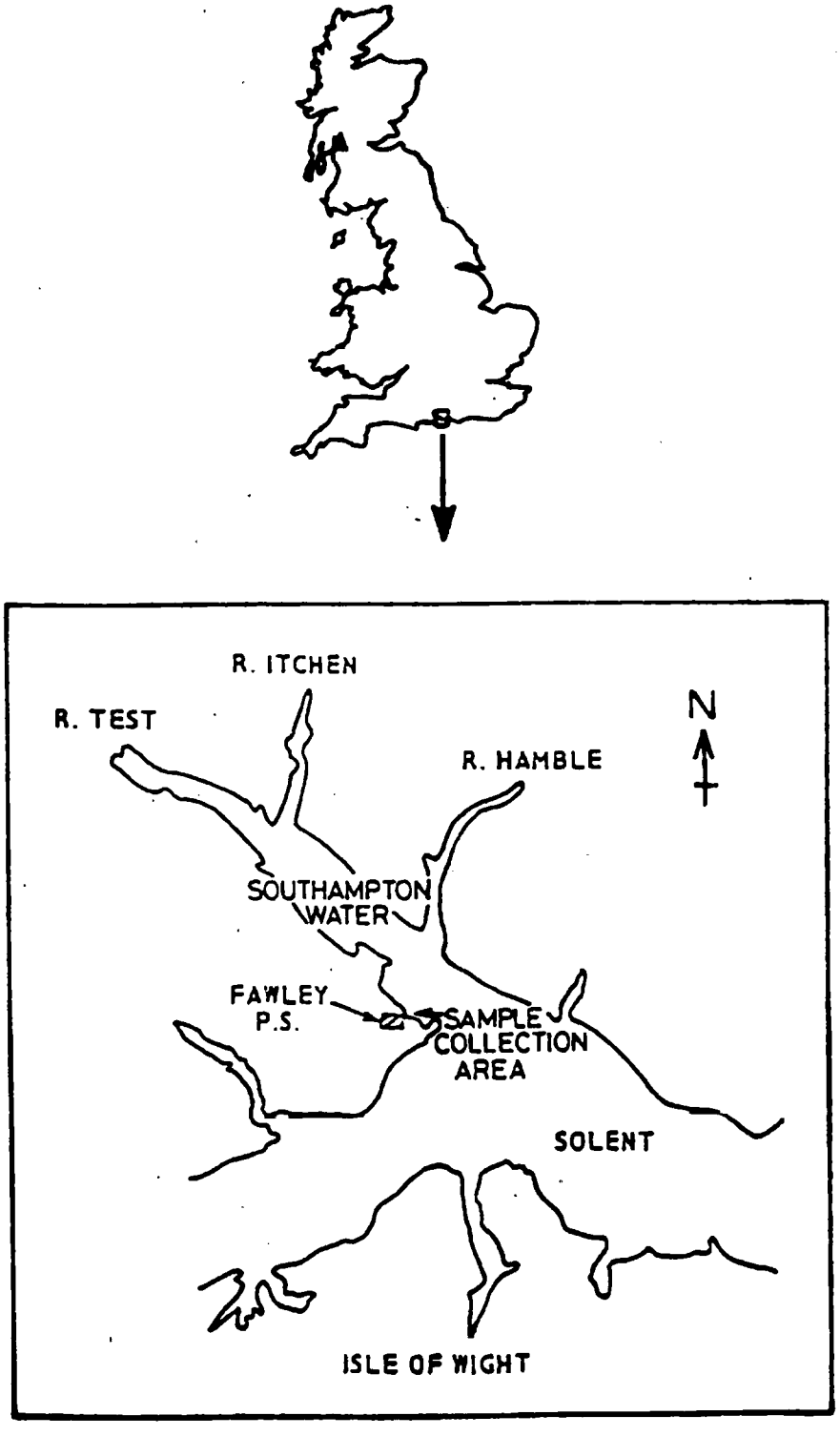
2.1 Introduction

The ecology of an estuary cannot be considered in isolation from the physical factors that may influence it, such as salinity, temperature, tidal regime and bottom type. These factors depend on the underlying hydrography, physical geography and geology. Additionally, in Southampton Water, artificial factors arising from the economic development of the estuary must also be considered.

2.2 Southampton Water

Southampton Water (Lat. 50° 49' N, Long. 1° 18' W) is a drowned river valley on the south coast of England (Figure 1). It is a roughly linear body of water approximately 10 km long and 2 km wide which forms a north-westerly extension of the central Solent. Because the main axis of Southampton Water is perpendicular to the prevailing south-westerly winds, the estuary has been used for centuries as a sheltered haven for shipping. During the last century Southampton Water has been progressively deepened to accommodate commercial shipping. Today the shipping channel is dredged to a depth of about 13 m below mean tide level, permitting access to the vast majority of ships; indeed, it is the world-wide shipping that visits the port of Southampton that is thought to be responsible for many of the exotic species found in Southampton Water (e.g. *Styela clava*). The western shore of Southampton Water is further protected from southerly winds by Calshot Spit so that, although there are variations in the wave climate depending upon prevailing weather conditions, the estuary around Fawley Inlet is substantially free from severe wave action.

FIGURE 1 MAP SHOWING THE LOCATION OF SOUTHAMPTON WATER AND THE ASCIDIAN COLLECTION AREA



The shipping channel is bordered by broad intertidal mudflats giving way to shingle and sand on the eastern shore and to saltmarsh on the western. Considerable reclamation has taken place on the western shore. The immediate effect of replacing areas of sloping intertidal shore by vertical walls of steel or concrete which rise from very deep water was to reduce the habitat available to infauna (intertidal burrowing species) whilst greatly increasing the substrata available to fouling organisms that had hitherto been restricted to stones lying on the generally soft bottom. The new surfaces were rapidly colonised. They provided not merely a vast extension of epifaunal habitat, but also a vastly improved one as raising filter feeders even 0.5 m above the bottom results in faster growth and less mortality (see for example Walne, 1961). The long-term effect on the hydrography and ecology of the area of deepening and confining the water course is not known.

The unique tidal regime in the Solent, resulting from its position midway along the English Channel (and to a lesser extent from the presence of the Isle of White), gives over eight hours of rising or standing water with less than four hours of ebb (Webber, 1980). Within Southampton Water, complicated effects due to slack water coinciding with high or low tide, a double peak on the flood tide and a double high water at spring tides combine to produce a fast, short duration ebb flow and a slower longer flood flow, with a longer period of slack water at high tide, generating a relatively small tidal range and weak tidal streams. Consequently, the sub-littoral zone is exposed for a shorter period than for estuaries with more symmetrical tidal regimes.

In general, salinity declines from a maximum of 34‰[1] at the seaward end of Southampton Water, the salinity at any point depending on the state of tide and degree of mixing. Southampton Water is a partially mixed estuary (Dyer, 1970) but its salinity structure depends

[1] UNESCO Technical Papers in Marine Science No 36 (1981) and No 45 (1985) established the international system of units (SI) in oceanography. Salinity is defined as a pure ratio to be presented without symbol or indicator of proportion (e.g. ‰, ppt, mg l^{-1}, g kg^{-1}). The author is unsure whether this change has been recognised sufficiently for it to be implemented in a non-specialist document. Consequently to avoid perplexing the reader I have retained ‰ as a recognisable salinity symbol.

very much on tidal state and, to a lesser degree, the seasonal cycle in freshwater flow. At high water, salinity in Southampton Water exhibits little evidence of stratification and summer surface salinity normally exceeds 30‰ as far as the confluence of the Test and Itchen estuaries. Some stratification occurs in both the Test and Itchen arms of Southampton Water, but the water is generally well mixed towards the mouth of the estuary. However, at low water the surface salinity at Calshot may fall below 30‰ in the wetter months and stratification may develop, with vertical differences ranging from 0.5 to 2‰ (Westwood, 1980).

The principal sources of fresh water are the Rivers Test and Itchen, producing a combined discharge of about 16 m^3 s^{-1} with fairly steady flows throughout the year (M.H.L.G.,1967). Although this represents only a very small proportion, approximately 1.3%, of the neap tidal prism, this fresh water input can affect water temperature as well as salinity within the estuary. The upper estuary is colder in winter and warmer in summer than the open sea, but this variation is greatly attenuated by the time the water reaches Fawley where a similar, but smaller, effect is produced by shallow water draining off the extensive mud flats.

Concentrations of dissolved oxygen in Southampton Water rarely fall below 80% saturation and may exceed 150% during algal blooms (Phillips, 1980). Concentrations of dissolved and particulate organic carbon generally vary within the ranges 1.0 - 3.0 mg l^{-1} and 0.2 - 1.0 mg l^{-1} respectively with higher levels of particulate organic carbon during rough weather, probably due to re-suspension of sediments, and during algal blooms (Collins, 1978); dissolved organic carbon concentration declines seaward down Southampton Water (Moore, 1978). Levels of nutrients and organic material are high in the estuary (Collins, 1978) as it receives effluent, treated to varying degrees, from sewage works serving a population of about 300,000. The estuary also receives industrial discharges from a large oil refinery and associated petrochemical complex.

The substrate within Southampton Water is mud, with varying amounts of sand. The 'typical' littoral zone has a steep shingle bank at about the high water mark, giving way to a band of pebbles, small rocks and stones, and a gently sloping mud flat that extends to the low water mark and beyond into the sublittoral zone. Extensive areas of shell debris and shingle occur on the surface of all mud flats. The majority of substrata available for larval settlement has been introduced by man, and takes the form of tidally exposed (fixed) substrata such as dock walls, piers, jetties and dolphins, and floating (constant depth) substrata such as pontoons and buoys.

2.2 Fawley intake.

The cooling water intake for the 2000 MW oil-fired power station at Fawley is situated in a small inlet on the west bank of Southampton Water (Figure 1). The inlet consists of a dredged central channel, about 7 m deep at high water, flanked by extensive mudflats (Plate 1).

The power station pumps up to 63 $m^3 s^{-1}$ of cooling water (salinity 30-34‰) from Southampton Water via the channel, which produces an essentially unidirectional flow close to the cooling water intake. Water is drawn under an oil-boom and along a concrete channel (Plate 2) to screens and pumps. The cooling water pumps are situated below low tide level so that the greatest intake water velocity is found sub-surface. The enhanced velocity is rapidly attenuated so it is doubtful whether this effect could be transmitted as far as the oil-boom. However, it is augmented by the drag of the concrete channel sides and the 1 m deep skirt of the oil-boom itself. These factors combine to produce a velocity-depth profile with the maximum water velocity occurring at just over 1 m depth around the sampling area. The singular direction of flow and velocity profile of water in the channel render the flow characteristics of the inlet very unusual and may have some bearing on the distribution of the local ascidian populations.

Plate 1 **Fawley inlet and Southampton Water**

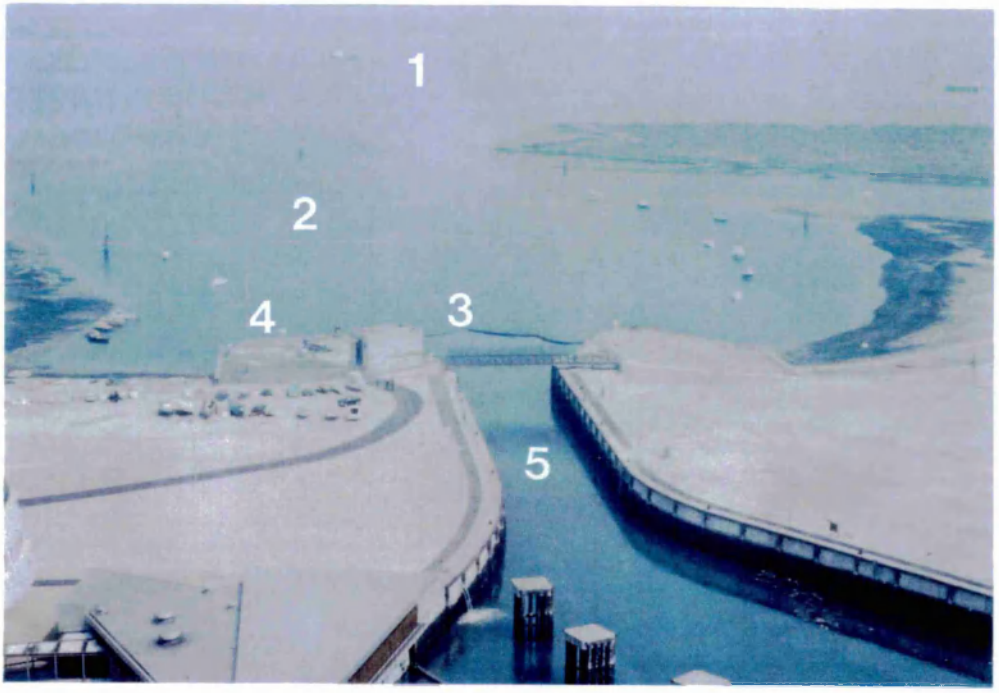
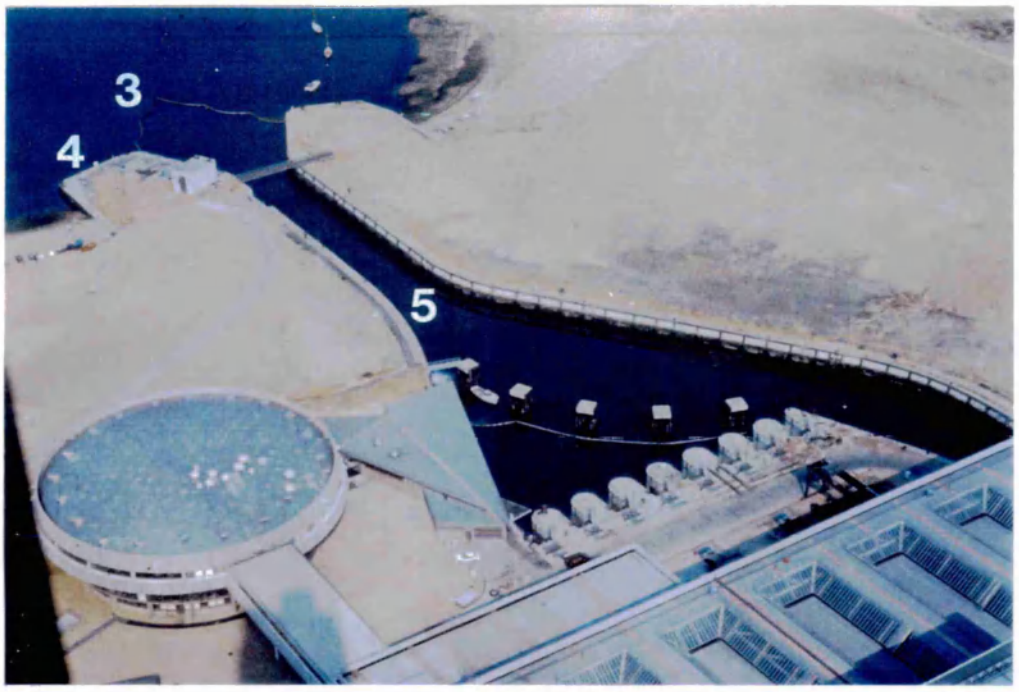


Plate 2 **Fawley Power Station cooling water intake**



Key

1 Southampton Water
2 Fawley inlet
3 Oil boom
4 Site of pontoon
5 Cooling water inlet

Spawning stock collection and field experiments took place in Fawley Inlet at the entrance to the cooling water intake. All ascidians were collected from the area just seaward of the oil-boom. *Ciona intestinalis* and *Ascidiella aspersa* were collected from the ropes and chains anchoring the oil-boom and nearby mooring buoys. Some *C. intestinalis* were collected from the base plate of a submersible pump in the same area. A few *S. clava* were collected from the oil-boom mooring ropes, but the great majority came from an essentially monospecific population growing on the sides and bottom of a floating pontoon adjacent to, and seaward of, the oil-boom. Fouling panels and settlement ropes were suspended from the shoreward edges of the oil-boom. Sub-surface larval sampling was carried out from the dockside just station-side of the oil-boom.

2.3 Water quality

Successful survival of filter-feeding animals depends in part upon the supply of organic detritus and the short-term stability of environmental conditions. Summer concentrations of between 30 and 55 mg l^{-1} suspended solids have been recorded in Fawley intake water, with the maximum concentration occurring at low water (Figure 2) when the flanking mudflats are draining, and a secondary peak at high water after the rising tide has scoured the mudflats (Davis, 1983a). The environmental parameters most likely to vary in an estuarine ecosystem are salinity and temperature. At Fawley, salinity varies by about 2‰ through the tidal cycle; it varies with, but generally lags, tidal height (Figure 2). Temperature varies by up to 1°C through the tidal cycle in winter and 2°C in summer (Davis, 1983a), the winter minimum and the summer maximum occurring when the mudflats are draining. Thus these parameters are stable over the short-term.

The period for reproduction and growth of sedentary filter-feeding organisms in the Fawley intake channel will depend greatly on water temperature and food availability. Water temperature

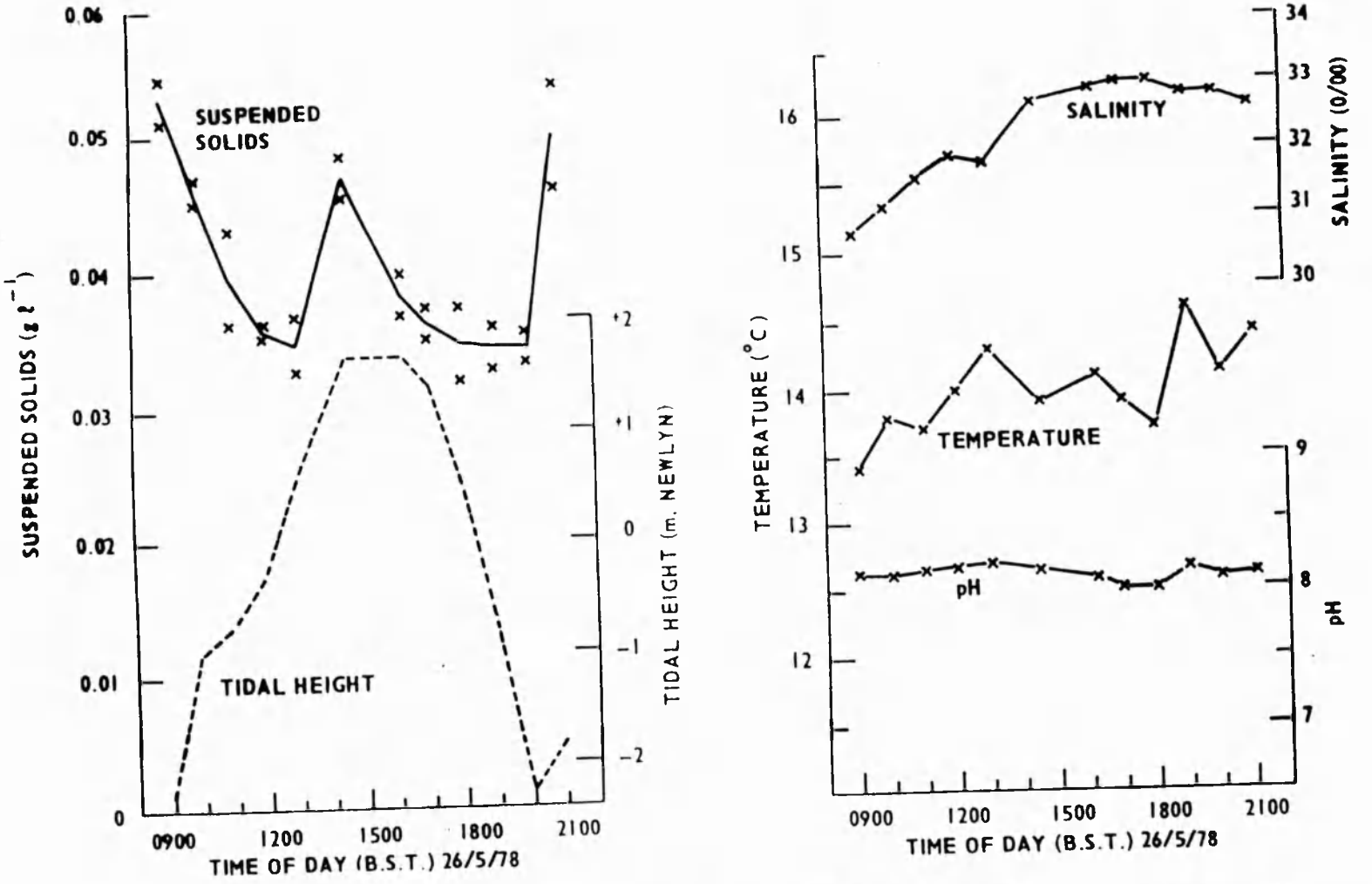


FIGURE 2 **THE VARIATION OF SUSPENDED SOLIDS, SALINITY, TEMPERATURE AND pH THROUGH A TIDAL CYCLE AT FAWLEY POWER STATION INTAKE**

(from Davis, 1983a)

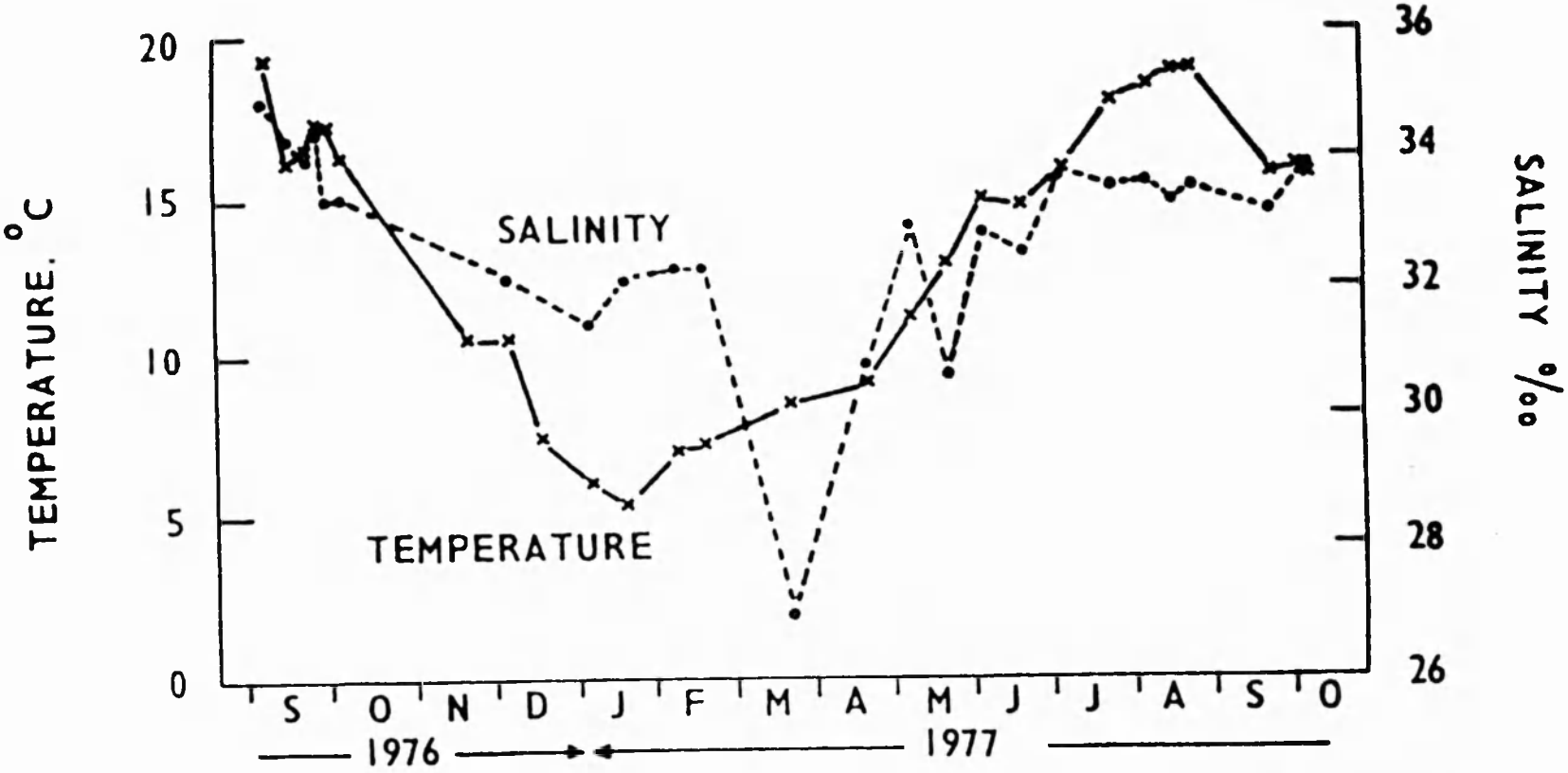


FIGURE 3 SEASONAL VARIATION IN THE TEMPERATURE AND SALINITY AT FAWLEY POWER STATION INTAKE

(from Davis, 1983b)

typically exceeds 15°C from June to October (Figure 3). The concentration of organic detritus scoured from the mud flats can only increase when the productivity of the mud flats increases. In most estuaries the increase in mud flat productivity follows the "spring bloom", the explosive phytoplanktonic primary productivity increase which is followed by increases in zooplankton populations. In addition to detritus, most ascidians can use phytoplankton and zooplankton as food sources. However, in Southampton Water only a brief spring peak in primary productivity occurs (Figure 4); the maximum rate of primary productivity occurs in July, when temperatures are above 15°C (Davis, 1983b) and declines by early September. Chlorophyll *a* concentrations indicate that phytoplankton cells remain in the water column until early October (Figure 5) at Fawley. Copepod numbers at nearby Calshot peak in September (A. Hurst, pers. comm.).

The variation of illumination[2] through the water column was measured during the primary productivity studies referred to above (Davis, 1980). A field light meter was lowered into the intake channel and the light intensity measured at 0.5 m intervals. Two determinations were made during the month of May; one on a very bright sunny day, the other on a dull overcast day with light rain (Figure 6). Light intensity was measured in lux units and the spectral quality of the light was not determined.

This brief examination of the water quality experienced at the Fawley inlet suggests that conditions are favourable for lower estuarine filter-feeding organisms that reproduce between early July and late September. The species studied in this project are thought to spawn between early summer and late autumn.

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[2] The terms illumination and light intensity are commonly used by biologists, but criticised by physicists because the photopic sensitivity of the human eye is involved in the definition of the former (Jerlov, 1970) and a punctiform source of light in the latter. Irradiance, the energy or number of quanta per second falling on a surface from all directions, is a more precise parameter but is not easy to measure in the field. However, as downward irradiance was thought likely to be the most important component, and a high precision of light measurement was not required, light intensity (unidirectional) was measured.

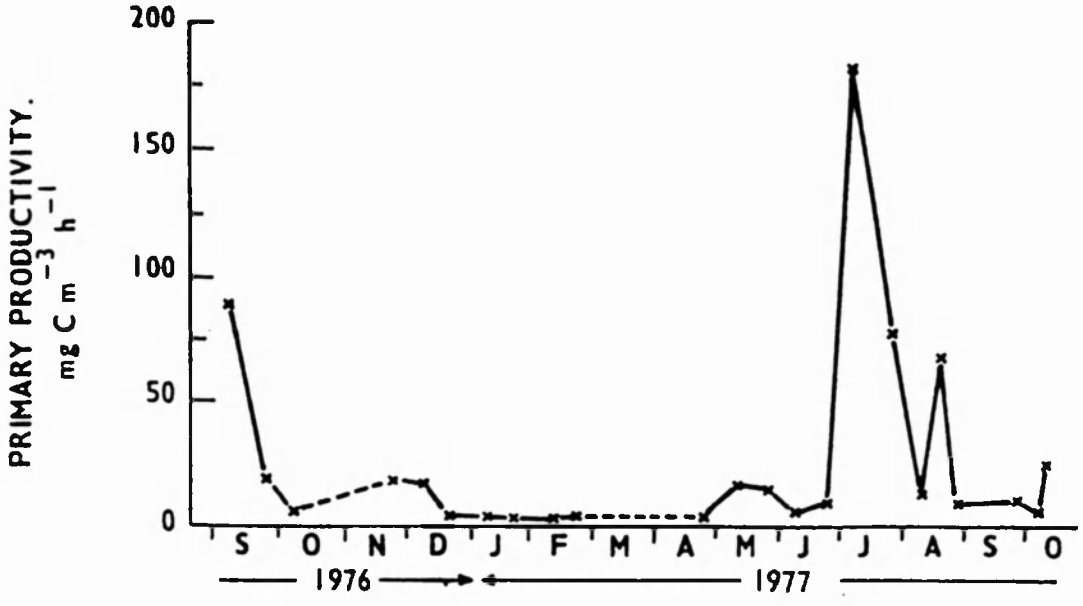


FIGURE 4 SEASONAL VARIATION IN PRIMARY PRODUCTIVITY AT FAWLEY POWER STATION INTAKE

(from Davis, 1983b)

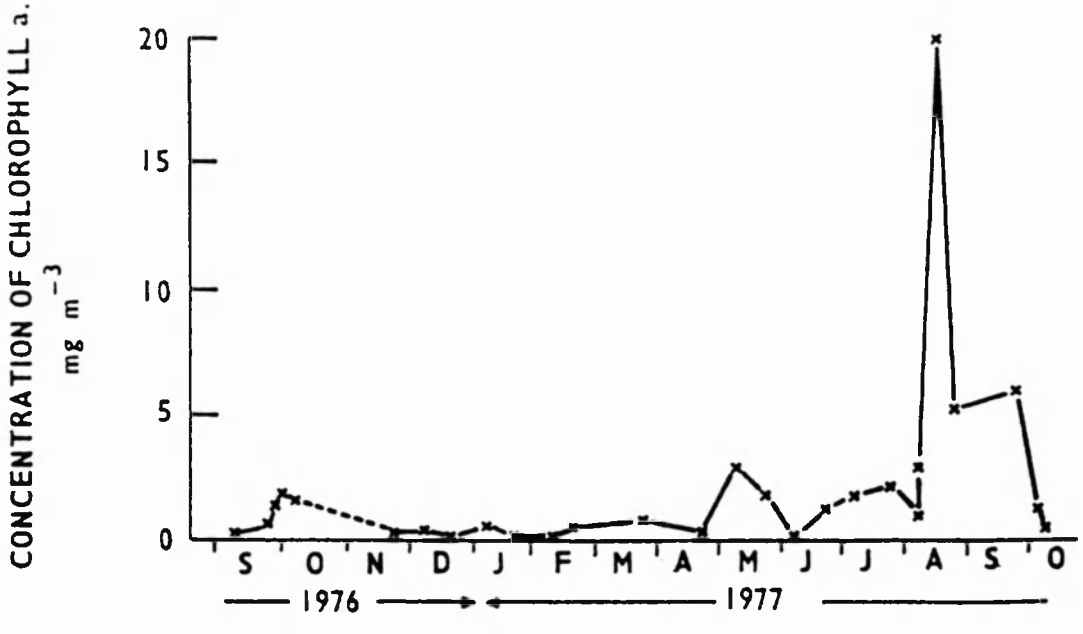
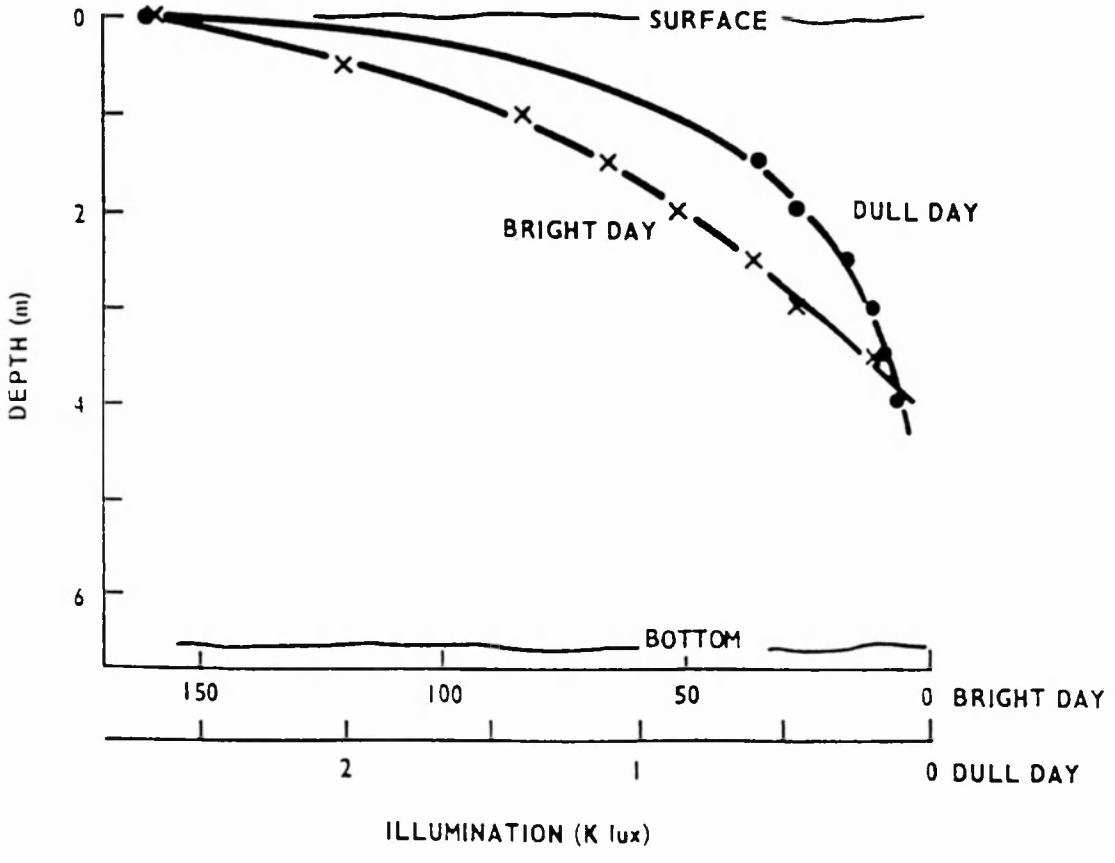


FIGURE 5 SEASONAL VARIATION IN THE CONCENTRATION OF CHLOROPHYLL a AT FAWLEY POWER STATION INTAKE

(from Davis, 1983b)

FIGURE 6 VARIATION OF ILLUMINATION WITH DEPTH AT FAWLEY POWER STATION INTAKE



(Taken from Davis, 1980)

CHAPTER 3 THE ORGANISMS STUDIED

3.1 General description of solitary ascidians.

Ascidians of the class Ascidiacea, within the subphylum Urochordata (= Tunicata) phylum Chordata, are marine sessile invertebrates which, by virtue of being chordate animals, are related to the vertebrates. The exact relationship is obscure, the chordate nature of ascidians being more apparent in the notochord and dorsal central nervous system of the motile larvae than in the sessile adult. Ascidians are wholly marine, with habitats ranging from mid-tidal level in the littoral zone to depths of over 6000 m in the abyssal zone (Bruun *et al.*, 1956).

Ascidian species can be grouped into two types, colonial and solitary. Colonial forms are composed of small zooids that are embedded in a common matrix, or joined by basal stolons, during some stage of their life cycle. The morphology of colonial ascidians is essentially two dimensional; they form flat patches on the substratum and can blanket other organisms. Solitary ascidians exist as clearly defined individuals, although they may grow as epizooites on other individuals. They adhere to a variety of solid substrates or, less commonly, anchor by means of a stolon to the sediment of soft bottoms. Solitary ascidians exhibit three dimensional morphology which renders them easier to collect and maintain in the laboratory. It should be noted, however, that the body of a solitary ascidian offers a large substrate area for epibiont settlement which can present problems when culturing larvae if the epifauna present consume or produce eggs and/or larvae. It is essential to remove all epifauna, particularly colonial ascidians, from the adult stock if monospecific larval populations are required for experimentation, to avoid time consuming identification of individual larvae, because the larvae of most ascidian species look very similar under low magnification - they are tadpole shaped and motile.

All of the solitary ascidians found in shallow water are filter-feeding animals. Water is transported through the animal by cilia associated with the branchial basket. It is drawn in through the oral siphon into the branchial sac where phytoplankton and organic detritus are filtered out. Food particles are trapped in mucus secreted by the endostyle and transported across the branchial wall to the oesophagus. Processed water is expelled from the atrial siphon. The organisms will thus survive successfully in environments with high productivity and/or high organic detritus levels, and can easily be maintained in the laboratory in flowing sea water.

The three species studied in this project reproduce sexually. In Southampton Water *Ascidiella aspersa* spawns in early summer and *Styela clava* in the autumn; the breeding season of *Ciona intestinalis* overlaps that of the other two species. All three species are oviparous, shedding their eggs and sperm into the water where fertilisation occurs and development continues. Tadpole shaped larvae hatch up to a day after spawning, depending upon the species. Thus culturing fertilised eggs from a single species through to hatching is the simplest method of obtaining a supply of monospecific larvae. However, the majority of ascidian species produce free swimming larvae (the main exceptions being in the family Molgulidae), so it is important that there is only one species in the spawning stock if identification of individual larvae is to be avoided.

The tadpole larva consists of a trunk or "head" which is 150-250 μm long and 100-125 μm wide, and a slender muscular tail about 750 μm in length. The whole larva is covered with an extracellular tunic or test, which is flattened to form a fin around the tail. The tunic is acellular, but a number of highly vacuolated extra-embryonic follicle cells derived from the oocyte may adhere to the outer surface; these are termed test cells and appear to be responsible for fin formation (Robinson, *et al.*, 1986). There are pairs of ciliary sensory cells lying along the mid-line of the tail epidermis, but it is not known whether these provide sensory input about aspects

of the outside environment or merely indicate tail orientation; Torrence & Clony (1982) suggested that these cells were mechanoreceptors which might trigger swimming.

There are a set of (non-eversible) epidermal, adhesive papillae at the anterior end of the head. Secretion of adhesive by these organs enables the settling larva to adhere to the substrate (Lane, 1973); anchoring of the larva is the first event of metamorphosis (Clony, 1978). Larvae of solitary ascidians rarely have obvious ampullae (secondary organs of attachment). The head houses the cerebral vesicle, which is the anterior portion of the central nervous system, and rudiments of the adult body. The cerebral vesicle generally contains two sensory structures thought to control swimming behaviour and orientation; a light-sensitive ocellus and a gravity-sensitive statocyte containing a statolith (sometimes referred to as an otolith), combined as a photolith in some species. A possible third sensory structure has been found in phlebobranchs and stolidobranchs (Dilly, 1969; Eakin & Kuda, 1971; Svane, 1982) the function of which is unknown; Eakin & Kuda, (1971) suggested that it might act as a hydrostatic pressure receptor. The remaining part of the central nervous system extends within the tail, dorsal to the notochord.

The tadpole larva is peculiar to ascidians. The larva is always enclosed in a tunic that occludes the siphons, making feeding impossible. Thus all ascidian larvae are lecithotrophic, and remain incapable of feeding until after metamorphosis has commenced. The larvae of solitary ascidians have the rudiments of the prospective juvenile organs within their trunk sections. The development of these organs ceases at hatching and the rudiments remain relatively undifferentiated until after the onset of metamorphosis, so the larval phase is not a developmental period. The function of the larva appears to be dispersal, but it may also be able to exercise some control over the selection of a settlement site (Lambert, 1968; Cloney, 1978: Young & Braithwaite, 1980; Cloney, 1987). All ascidian larvae move by flexing muscles in the tail. The

larva swims by "twitching", short bursts of swimming activity in which it may travel up to ten body lengths per second (Bone, 1992); for solitary ascidians, larval body length ranges from 0.6-1.5 mm. The time spent in this dispersive stage may vary from minutes to many hours, depending on the species and environmental conditions. Various patterns of larval movement prior to settlement have been reported but, with the exception of the tendency to swim upwards shortly after hatching, larval movement during the pelagic phase appears to be species dependent.

3.2 *Ciona intestinalis* (Linnaeus)

3.2.1 Description of the adult

Ciona intestinalis is a fast growing solitary sea squirt. It is tall, with a soft gelatinous cylindrical body (Plate 3) which retracts significantly when disturbed. The test is partially transparent and is rarely fouled with epibionts. The animal varies in colour from greenish-yellow to orange, with orange or yellow bars on the branchial sac. It is attached at one end to the substratum; the oral and atrial siphons, which are at the other end of the body, are conspicuous and the margins of the siphons have characteristic scalloped yellow markings. A full description can be found in Millar (1953). Specimens collected during this study ranged from 5 - 10 cm in length.

3.2.2 Description of the larva

The larva is a streamlined tadpole shape (Plate 4) consisting of a thickened anterior trunk and a narrow posterior tail with an overall length ranging from 0.98 to 1.05 mm. It is covered by a transparent tunic or test which is expanded dorsally and ventrally into a wide fin running the length of the body and projecting beyond the end of the tail. A few scattered cells lie in the test.

Plate 3 **Adult specimens of *Ciona intestinalis* (x 0.7)**

Plate 4 ***Ciona intestinalis* larvae showing tunic and papillae (x 100)**

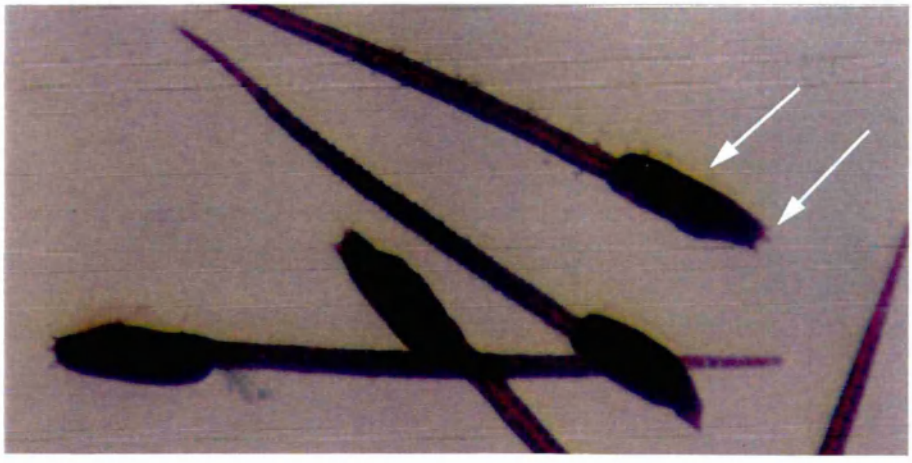


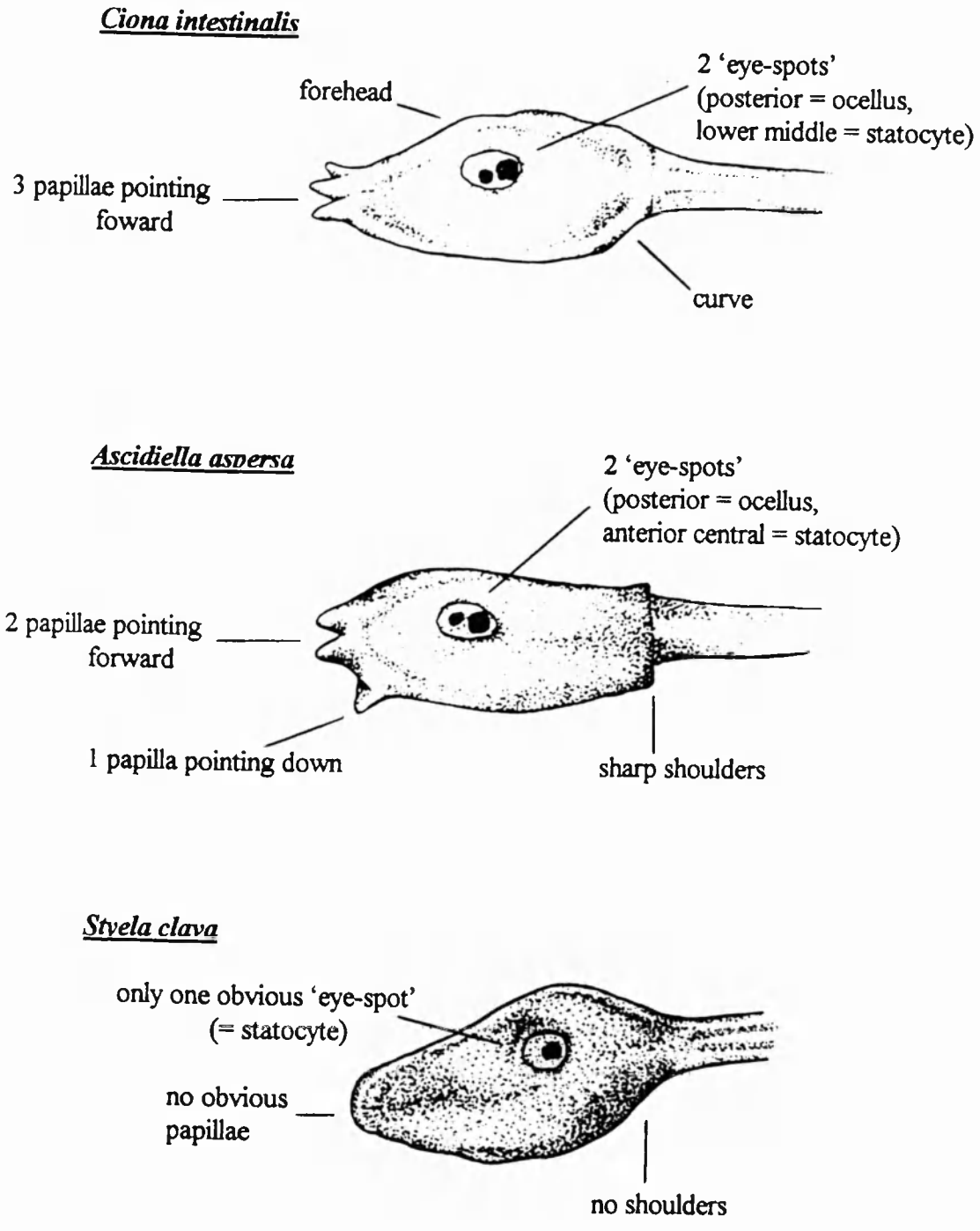
Plate 5 ***Ciona intestinalis* larvae showing the statocyte and ocellus (x 100)**

The trunk contains the prosencephalon, the bulbous anterior end of the central nervous system. This contains a single large ventrical or sensory vesicle, situated midway along the trunk. Two cells containing black melanin pigment (Minganti, 1957) are located on the wall of this vesicle (Plate 5) and these are differentiated as sense organs - the statocyte (or otolith) and the ocellus (Berrill, 1947). The statocyte contains a single large (10-15 μm diameter) pigment granule or statolith (Whittaker, 1966) and is concerned with balance and the detection of gravity (Dilly, 1963; Eakin & Kuda, 1971; Katz, 1983). The ocellus occupies the postero-dorsal portion of the sensory vesicle and consists of 70-80 tiny pigment granules in the form of a shallow cup, the cavity of which holds three lens cells (Whittaker, 1966). Behind the pigment cup is a layer of retinal cells (Dilly, 1964; Eakin & Kuda, 1971; Katz, 1983). The ocellus is involved in the phototactic reactions of the larva. The pigmented areas appear to be of similar size.

The posterior two-thirds of the dorsal surface of the trunk is flat, the anterior third is curved and a slight brow separates the two sections. Dorsally the line of the trunk continues into the tail, but the ventral line curves from the anterior end and rises sharply to meet the tail (Figure 7). The anterior end of the trunk bears one median-ventral and two dorso-lateral papillae in a triangular arrangement (Katz, 1983), by means of which the larva attaches itself to a solid substratum before metamorphosis. All three papillae project forward (Plate 4).

In the laboratory, newly hatched larvae lie on the bottom of the container and move in circles by flicking their tails to one side only. After 15 to 30 minutes they swim up and hold station close to the surface; swimming consists of short bursts of activity with rest periods of several seconds. The larvae rotate around their longitudinal axis as they swim forward, producing a spiralling motion. They remain free swimming for 6-36 hours (Berrill, 1950).

FIGURE 7 **THE MAIN DISTINGUISHING FEATURES OF THE LARVAE OF THE THREE ASCIDIAN SPECIES**



(Drawn from stained preserved specimens)

3.2.3 Distribution

C. intestinalis is a cosmopolitan species. It occurs in the Mediterranean, the western seaboard of Europe, off the coasts of North and South America, Australia and Japan. It is widely distributed around the British Isles. It occurs from the tidal zone down to depths of about 500 m (Millar, 1953), and is tolerant of a wide temperature and salinity range (Harant & Vernières, 1933). It is an opportunistic coloniser, rapidly settling on recently exposed surfaces. It is often abundant on man-made structures, in sheltered areas with some current. The breeding season in British waters may extend from April to September (Millar, 1953) but is probably greatly influenced by local temperature conditions. It has a relatively short life-cycle (less than a year). Individual populations show tremendous fluctuations in numbers.

3.3 *Ascidiella aspersa* (Müller)

3.3.1 Description of the adult

Ascidiella aspersa is a tall solitary sea squirt usually found in clumps and attached to the substratum by its base (Plate 6). The body is oval with a fluted oral (inhalent) siphon at the top and an upward-directed atrial (exhalent) siphon one third of the way down the side of the body. The test has a cartilaginous consistency; it is grey and partially transparent, often encrusted with compound ascidians and other epibionts, with adhering detritus. There is a series of lighter marks around the edge of each siphon. Adult specimens were identified using Millar (1969; 1970).

There is little to distinguish *A. aspersa* and *A. scabra*, which led to some confusion in the early literature (Lindsay & Thompson, 1930). One major distinguishing feature is the size of the egg.

The outer follicle cells of *A. aspersa* eggs are several times the diameter of the inner test cells, whereas those of *A. scabra* are about the same size as its test cells (Lindsay & Thompson, 1930). These large follicle cells produce a large diameter egg which distinguishes *A. aspersa* from all other species belonging to the family. Berrill (1928) reported the diameter of *A. aspersa* eggs from English Channel adults as a minimum of 0.4 mm whereas *A. scabra* eggs had a diameter of approximately 0.2 mm. A fertilised egg of the species used in this study is shown in Plate 7; its size indicates that the species is *A. aspersa*. Furthermore, *A. scabra* grows to a maximum length of 4 cm whereas *A. aspersa* grows to 12 cm long (Lindsay & Thompson, 1930). The specimens collected during this project ranged from 5-8 cm in length, supporting the assertion that the species used in the study was *A. aspersa*. A full description of *A. aspersa* is given in Lindsay & Thompson (1930) and Berrill (1950).

3.3.2 Description of the larva

The larvae of *A. aspersa* are slightly shorter than those of *C. intestinalis*, ranging from 0.95 to 0.98 mm, but the trunk is much thicker and bullet shaped with curves to dorsal and ventral surfaces (Plate 8). The trunk contains a sensory vesicle situated approximately one third of the distance along the trunk. This vesicle contains two black pigmented sense organs, one at the centre and the other (larger) on the posterior edge of the vesicle (Plates 9 &10). There are three papillae on the anterior end of the trunk, two dorso-lateral and one median-ventral as in the *C. intestinalis* larva. However, the papillae of the *A. aspersa* larva are thicker and the median-ventral papilla points down at an angle of approximately 60° to the body line, producing a characteristic "spur" (Plate 11).The tail appears to emerge centrally from the trunk, producing sharp shoulders at both dorsal and ventral junctions (Figure 7). These attributes render the larvae of *A. aspersa* readily distinguishable from that of *C. intestinalis*.

Plate 6 **Adult specimens of *Ascidiella aspersa* (x 0.8)**

Plate 7 **An egg of *Ascidiella aspersa* (x 50)**

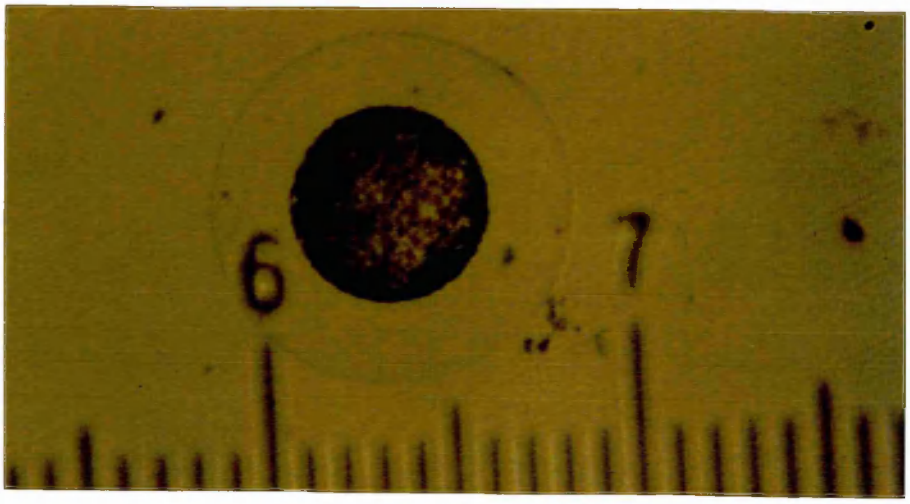


Plate 8 ***Ascidiella aspersa* larvae showing tunic and papillae (x 75)**

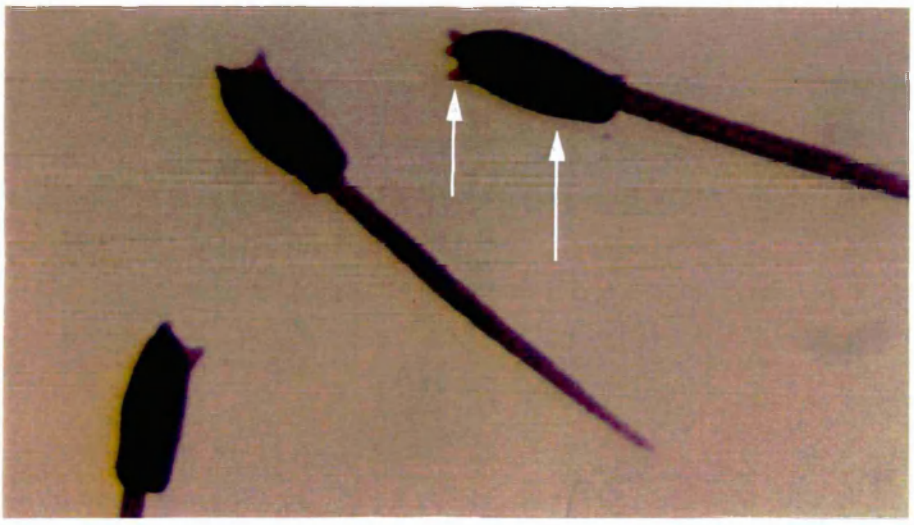


Plate 9 ***Ascidiella aspersa*** **larvae showing sense organs (x 75)**

Plate 10 ***Ascidiella aspersa*** **larvae showing sense organs (x 100)**

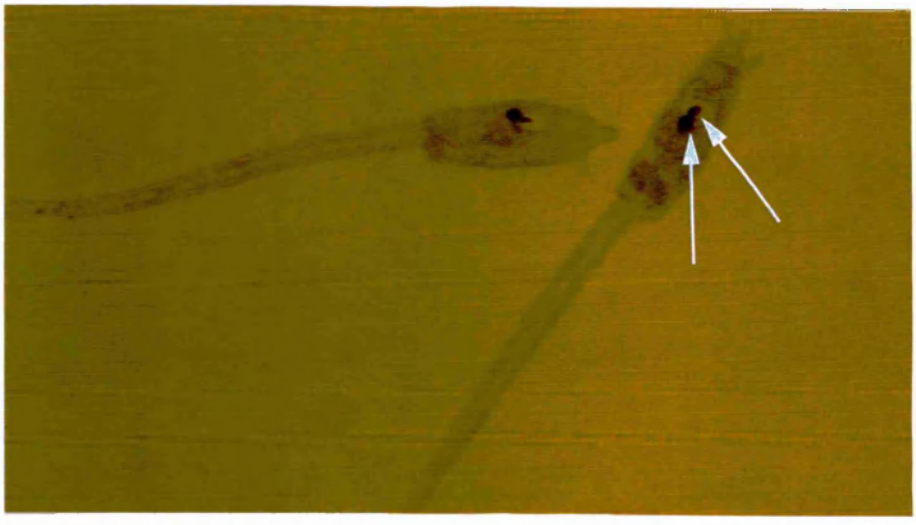
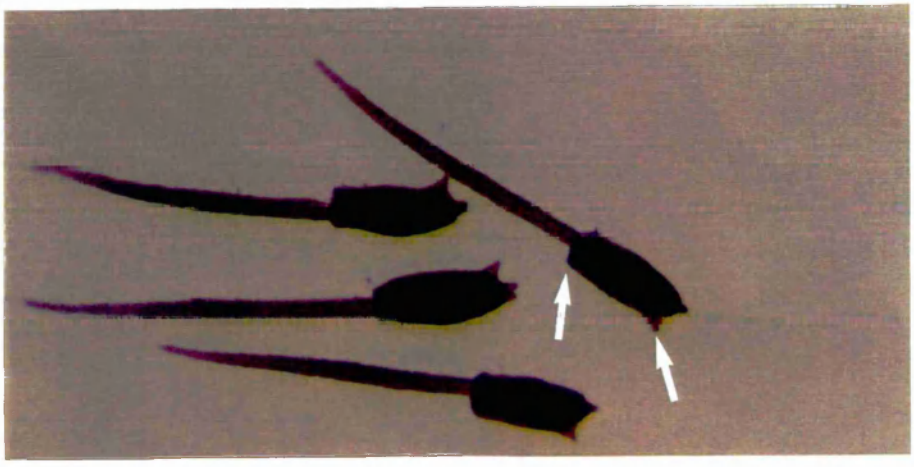


Plate 11 ***Ascidiella aspersa*** **larvae showing characteristic spur papilla and sharp shoulders (x 75)**



3.3.3 Distribution

The distribution of *A. aspersa* is a little uncertain because of the occasional lack of distinction between it and *A. scabra* in the older records. However, it appears that it occurs throughout the Mediterranean, on both sides of the English Channel and all around the British Isles. It is usually found in shallow sheltered sites, such as harbours, attached to shells or pebbles on mud or on silty rock; it was abundant on mooring ropes in the Fawley inlet. It is not found at depths exceeding 50 m (Berrill, 1950).

3.4 *Styela clava* Herdman, 1882.

3.4.1 Description of the adult

The solitary ascidian *Styela clava* is attached to the substratum by a short narrow stem-like stolon, which usually represents about one third of its total length. The stolon renders it readily distinguishable from other British solitary ascidians (Plate 12). The base of the stolon is attached to the substratum by means of an expanded membranous plate, termed a hapteron. The adult is large, specimens 15 cm in length were commonly collected during this project. It has a thick leathery tunic which is distinctly mammillated. The yellowish-brown body is elongated, shaped like an Indian club, with two terminal openings. The body and stolon are longitudinally pleated and the stolon is often twisted, terminating in the hapteron. On the upper half of the body the pleats are overlain by folds and mammillations (swellings) which become more pronounced towards the anterior (free) end. These mammillations are generally creamy-yellow in colour, but always lighter in colour than the reddish-brown depressions. The body texture is firm and the tunic tough.

Plate 12 Adult specimens of *Styela clava* (x 0.8)

Plate 13 *Styela clava* larvae showing sense organ (x 100)

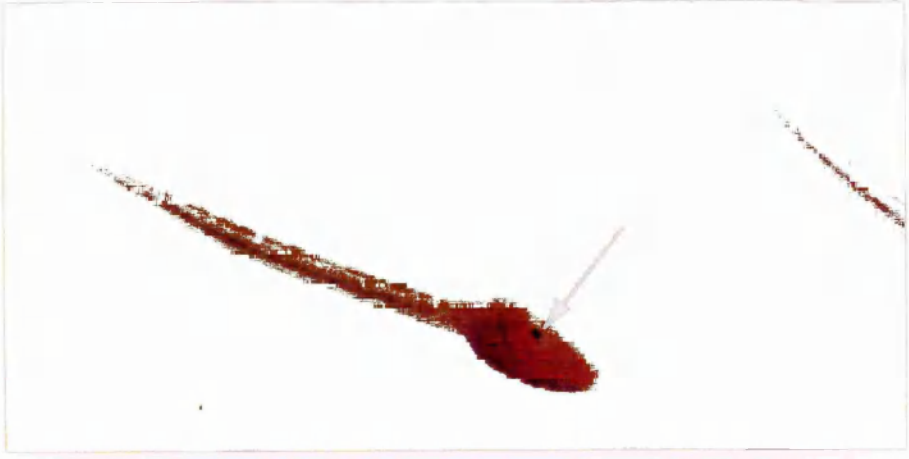
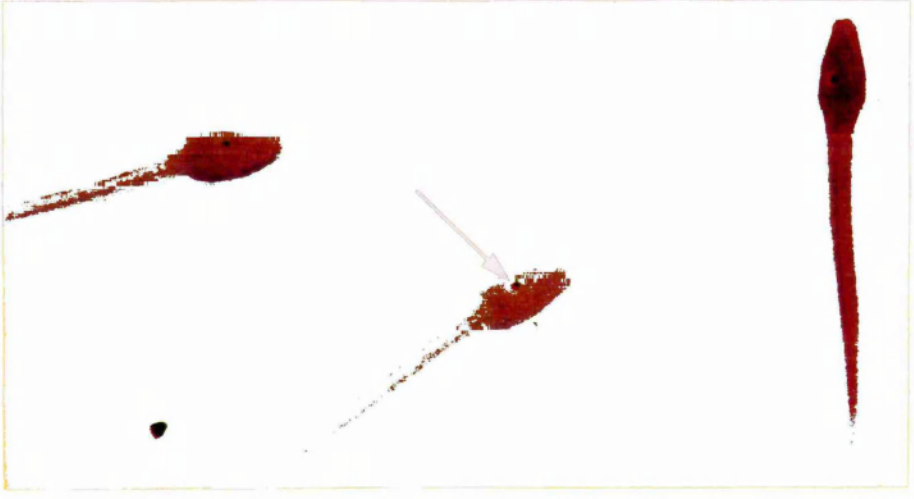


Plate 14 *Styela clava* larvae showing sense organ (x75)



The oral (inhalant) siphon is terminal and the atrial (exhalant) siphon is set close by on the true dorsal surface. Both siphons are raised and tapered; they are distally marked with four chocolate-brown stripes that alternate with four narrower, pale stripes. Each of the four brown stripes is subdivided by a central paler zone which does not quite reach the edge of the siphon. Adult specimens were identified using Millar (1969; 1970). More detailed, illustrated descriptions of the species are to be found in Carlisle (1954) and Wallace (1961).

3.4.2 Description of the larva

The larvae of *S. clava* are shorter than those of the other two species described, ranging from 0.83 to 0.87 mm. The trunk is similar in proportion to that of *A. aspersa*, but the anterior end is square and possesses no obvious papillae (Plates 13 & 14). Dorsally the body line rises linearly to a "brow" at approximately two thirds the length of the trunk, then curves down into the tail. The ventral line is a smooth curve from anterior end to the tail (Figure 7). The sensory vesicle is situated slightly more than half-way along the trunk and contains a large statocyte, a single cell distended by a mass of black pigment, and a relatively small ocellus (Wallace, 1961). The ocellus consists of a single lens cell and a single pigmented retinal cell; it is extremely difficult to distinguish from the statocyte so that there only appears to be one pigmented spot. The larvae of most styelids have either simplified ocelli or compound sensory structures that incorporate elements of both the ocellus and the statocyte into a single structure (Grave, 1944; Whittaker, 1966; Svane & Young, 1989). There are three papillae at the anterior end of the trunk that are difficult to see except under high magnification (Holmes, 1968); under a dissection microscope there appear to be no papillae on the square anterior end of the trunk and this is an extremely useful identification characteristic.

3.4.3 Distribution

S. clava is native to the north west Pacific, particularly Japanese waters, the Sea of Okhotsk and the coasts of Korea and Siberia. It appeared in Californian waters in 1932 (Abbot & Johnson, 1972). It was first recorded in British waters in 1953 when six specimens were collected in the estuary of the Lynher River, near Plymouth (Carlisle, 1954). These specimens were initially designated *Styela mammiculata* sp. nov. by Carlisle, but Millar (1960) demonstrated that this "new species" was synonymous with *Styela clava* Herdman, 1882, first described in the Challenger Report (Vol. 17, 1882). It is probable that it was introduced into Plymouth Sound by military craft returning from the north-west Pacific after the Korean war in 1952. Holmes (1968) proposed that it was essentially annual in British waters; he reported that it breeds from late July to September and the young developed to full size and maturity by the following summer.

The water temperature regime in the north-western Pacific where *S. clava* originates is similar to that of the English Channel (Millar, 1960) so it has been very successful where it has become established; population densities of up to 200 adults m^{-2} have been recorded in the intertidal zone of Southampton Water (Holmes, 1968). It can be found on stones, walls and piles from about mid-tidal level down to at least 4 m below low water (Holmes & Coughlan, 1975), though individuals have been dredged from at least 10 m depth (Barnes *et al.*, 1973). It is absent from areas of consistently low salinity; it can survive exposure to a salinity of 8‰ (Kelly in Christiansen & Thomsen, 1981), but appears unable to tolerate even short periods of salinity below 5‰ (Holmes, 1971; 1972) which may occur locally after heavy rainfall. Conversely, it is intolerant of wave exposure, which limits its choice of full salinity sites. The cooling water inlet at Fawley Power Station offers high salinity sheltered water with moderate depth, and *S. clava* is abundant. Settlement on floating submerged substrata is usually close to the surface.

The distribution of *S. clava* can best be described as patchy. Since its initial discovery in Plymouth, it has appeared in Langston Harbour, Hampshire (Houghton & Millar, 1960); Southampton Water (Holmes, 1968); Milford Haven (Coughlan, 1969); Dieppe, France (Monniot, 1970); Cork Harbour, Ireland (Guiry & Guiry, 1973); Den Helder harbour, Netherlands (Huwae, 1974); Eastern Scheldt, Netherlands (Westerweel, 1975); Dinard, France (Huwae & Lavaleye, 1975); Ambleteuse, Netherlands (Buizer, 1980); Limfjord, Denmark (Christiansen & Thomsen, 1981); Heysham (Coughlan, 1985); Roscoff, France (Dauvin *et al.*, 1991) and Kattegat, Denmark (Lützen & Sørensen, 1993). A sheltered, high salinity site appears to be necessary for the initial population in any area; however, once established it rarely spreads any great distance to neighbouring suitable habitats. New colonies can generally be attributed to the inadvertent introduction by man rather than natural spread by dispersal of larvae.

Holmes (1968) observed that *S. clava* often appeared as a secondary settler and suggested that it preferred organic rich substrata. Lützen & Sørensen (1993) reported that it was commonly found attached to Japanese brown alga *Sargassum muticum*. Larvae sometimes settle on conspecific adults (Minchin & Duggan, 1988); during the present study young specimens were often found attached bud-like to the bodies of older ones, and occasionally three size ranges, one on another, were found attached to a single adult (suggesting a life span in excess of two years). However, the over-settling of existing biofouling by *S. clava* may merely be a function of its relatively late spawning season.

In British waters *S. clava* has no known predators nor appears to support commensals (Holmes, 1968), but copepods have been found in specimens (Minchin & Duggan, 1988). The tunic is attractive to epibionts, including barnacles, solitary and colonial ascidians, bryozoa and small algae - green, red or brown, depending on the depth at which the individual *S. clava* is living.

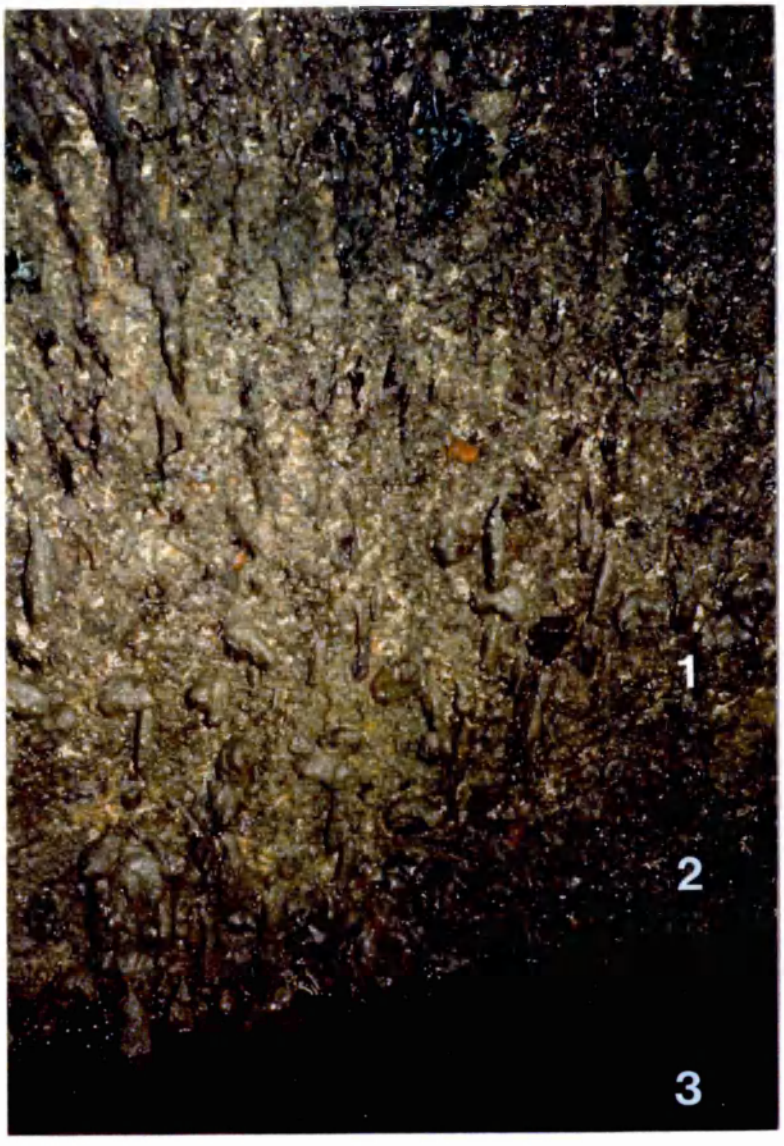
CHAPTER 4 THE PHENOMENON TO BE INVESTIGATED

4.1 Introduction

Ascidians growing on dock walls and other tidally exposed structures in the lower section of Southampton Water exhibit a degree of zonation (Plate 15). The uppermost zone starts at just above the spring tide low water level. Only species capable of withstanding some degree of desiccation and exposure to ultra-violet radiation, such as *Styela clava,* are found at this level. *S clava* may continue as the major species for the first metre or more of the permanently submerged substratum. Further down in the permanently submerged environment a dense population of *Ascidiella aspersa* is commonly encountered, and below this there is often a zone consisting of mainly *Ciona intestinalis*: these two zones often exhibit considerable overlap. The population density of *S. clava* may increase again below this depth. This zonation was alluded to by Holmes (1968,1971) and Holmes & Coughlan (1975) who observed that *S. clava* was the only solitary ascidian to be found above the low tide level in Southampton Water; below the low tide level *A. aspersa* became abundant, with *C. intestinalis* also appearing in great numbers. Specimens of *S. clava* attached to stones were observed by the author as high as the mid-tide level in Poole Harbour. Zonation can also be detected in the assemblages of fouling organisms recorded by Collins and Mallinson (1987) around the docks in the upper section of Southampton Water (Figure 8) and in the data reported by Mills (1984) for berth 101 of the docks.

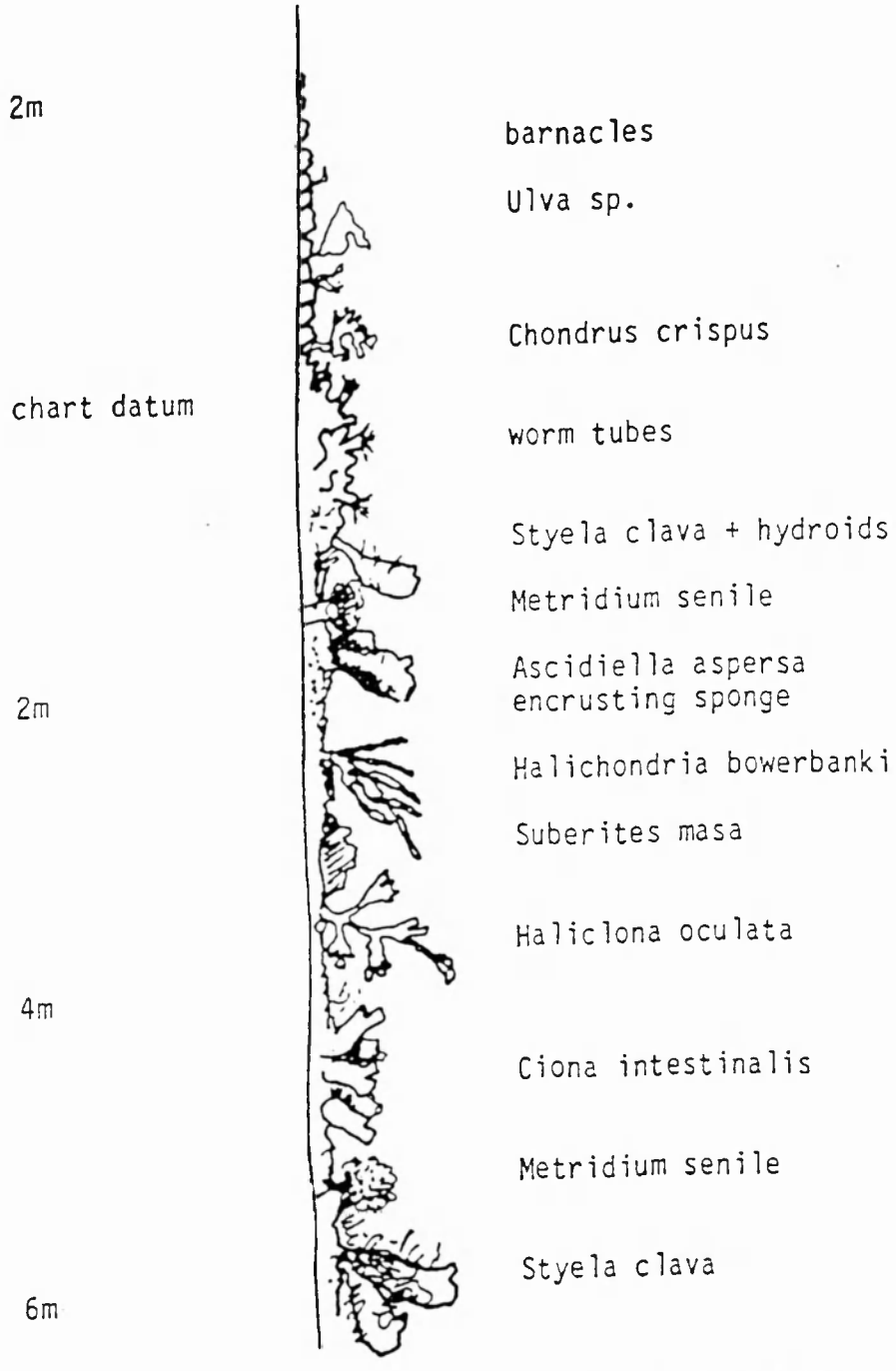
An examination of mooring ropes and chains in the area indicated that the three ascidian species were frequently zoned on constant-depth submerged substrata in the same order as on the tidally exposed dock wall, despite being removed from the selective pressure of occasional exposure. A typical example of a colonised mooring rope is shown in Plates 16-18.

Plate 15 **Ascidian zonation on Fawley dock wall**



Key
1 Occasionally exposed zone containing *Styela clava*
2 Rarely exposed zone containing mainly *Styela clava* with a few *Ascidiella aspersa*
3 Permanently submerged zone containing *Styela clava* and *Ascidiella aspersa* with a few *Ciona intestinalis*

FIGURE 8 **TYPICAL VERTICAL PROFILE OF A DOCK WALL IN SOUTHAMPTON DOCKS**



(from Collins and Mallinson, 1987)

Plate 16 ***Styela clava*** **attached to the surface end of a mooring rope**

Plate 17 ***Ascidiella aspersa*** **at mid depth on a mooring rope**

Plate 18 ***Ciona intestinalis*** **attached to the lower end of a mooring rope**

The section of the mooring rope closest to the surface was fouled almost exclusively with *S. clava* (Plate 16); a few *C. intestinalis* can be seen below the green seaweed. *A. aspersa* was dominant at mid-depth (2-3 m) with a few *S. clava* and *C. intestinalis* (Plate 17). It was not possible to examine the bottom of the mooring rope without diving, but the lowest part accessible from the surface at spring tide low water was fouled with mainly *C. intestinalis*; *A. aspersa* was frequent at this depth, and scattered specimens of *S. clava* were found (Plate 18).

Subsurface zonation was most apparent when a new substrate was being colonised; as the fouling community developed the zones tended to overlap. The "blurring" of zones was probably due to lack of available suitable substrate, degree of shading and other environmental factors. Nevertheless, in general, abundant populations of *S. clava* were found near the surface; for example, the vertical edges of a floating pontoon at the Fawley intake was fouled almost exclusively with *S. clava* from a depth of 5 cm (Plate 19), as were similar pontoons at Town Quay (at the mouth of the Test estuary), Fairey and Mercury marinas (lower Hamble estuary) and rafts in Poole Harbour. Holmes & Coughlan (1975) noted that *S. clava* was the dominant solitary ascidian species on buoys on the eastern shore of Southampton Water opposite Fawley, and was the only ascidian species found on the shore at Calshot (sea-ward of Fawley). *S. clava* was reported as the dominant fouling species on floating oyster-culture frames in Korean waters, accounting for 60% of the fouling biomass (Kang *et al.*, 1980), and on floating structures in the yellow sea (Chengxing, 1988). Lützen & Sørensen (1993) collected samples of *S. clava* that had attached to the underside of *Sargassum muticum* fronds floating on the surface, and noted that *A. aspersa* was growing on the fronds below the level of the *S. clava* population. However, the underside of objects floating on the surface of the Fawley inlet was not the exclusive province of *S. clava*; buoys and other horizontal surfaces at shallow depths were frequently found to support ascidian populations which included some specimens of *C. intestinalis* (Plate 20).

Plate 19 ***Styela clava*** **on a floating pontoon at Fawley**

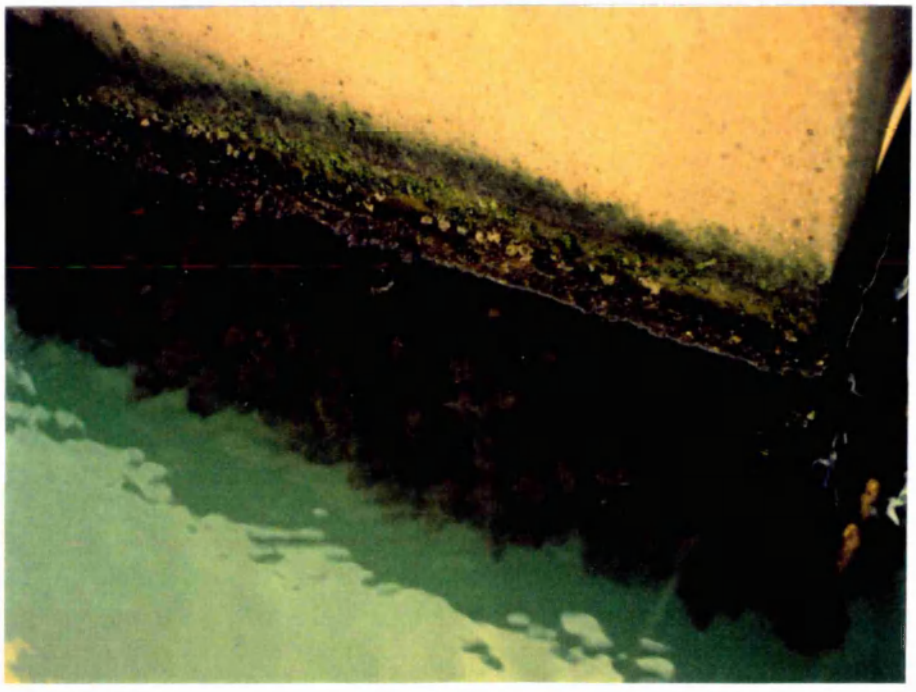


Plate 20 ***Styela clava*** **and** ***Ciona intestinalis*** **on an intake channel buoy**

Previous observations indicated that some ascidian communities that developed on constant-depth submerged substrata showed initial zonation. In particular *A. aspersa* exhibited an initial intense recruitment at a depth of about 2 m in August. Recruitment of *S. clava* was observed later in the year at depths of less than 0.5 m. The occurrence of these zones of settlement on submerged substrata at constant depth suggests that there may be zonation of some ascidian larvae in the water column. Before this phenomenon can be studied further, it is necessary to check that these observations are valid and repeatable.

Interference with the experimental substrata is a major problem with long term settlement studies of this type; consequently several attempts were required before an experiment could be carried through to a satisfactory conclusion. Three recruitment experiments were carried out between 1989 and 1992. In 1989 two sets of tufnol plates were suspended from the oil boom in the Fawley intake; one set was recovered and examined after 12 weeks, the other set was stolen before completion of the exposure period. The experiment was repeated the following year using two sets of asbestos plates. As previously, one set was recovered and examined after 12 weeks and, once again, the other set was removed before completion of the exposure period. The experiment was repeated and completed successfully in 1992.

4.2. Methods

4.2.1 Tufnol panels (1989)

Eyelets were fitted to both ends of two ropes (4 m length, 15 mm diameter) such that one end could be shackled to the oil boom at approximately water surface and a weight could be attached

to the other end. A Tufnol[3] panel (255 x 200 x 3.5 mm) was attached lengthways to each rope 5 cm below the surface attachment eyelet. A second panel was attached such that there was a gap of 58 cm between the top of this panel and the bottom of the first panel; the centre of this panel would subsequently be suspended 1 m below the surface. Two other panels were attached to each rope with 75 cm intervals between the top of the panel and the bottom of the previous one. The centres of these panels would subsequently be suspended 2 m and 3 m below the surface. All panels were previously sandblasted with No. 24 grit to reduce surface smoothness. All attachments were achieved by black cable-ties threaded through the rope and holes in the corners of the panels. The ropes with attached panels were suspended from the oil-boom in the intake channel of Fawley Power Station on May 7, 1989.

One set of panels was removed on August 6, 1989. The numbers of individuals of each of the three species of interest that had colonised each panel were counted and recorded. The second set of panels disappeared before completion of the experiment and no data are available on the numbers of ascidians recruited.

4.2.2 Asbestos panels (1990)

Asbestos panels were used from 1990 onward; this material has been shown to be a suitable substratum for the recruitment of marine sessile invertebrates (Butler, 1986). Eyelets were fitted to both ends of two ropes (4 m length, 15 mm diameter) as for the 1989 experiment. An asbestos panel (30 x 30 cm) was attached to each rope 5 cm below the surface attachment eyelet. A second panel was attached such that there was a gap of 50 cm between the top of this

[3] SRBP grade Tufnol was used because this material does not readily degrade, it provides a more uniform surface than natural substrates, it has a low surface energy (i.e. is easily wetted) and it is not selective with respect to settlement. It is the substrate used in standard fouling tests by the Admiralty.

panel and the bottom of the first panel; the centre of this panel would subsequently be suspended 1 m below the surface. Two other panels were attached to each rope with 70 cm intervals between the top of the panel and the bottom of the previous one. The centres of these panels would subsequently be suspended 2 m and 3 m below the surface. All attachments were achieved by black cable-ties threaded through the rope and holes in the corners of the panels. The ropes with attached panels were suspended from the oil-boom on May 26, 1990.

One set of panels was removed on August 19, 1990. The numbers of individuals of each of the three species of interest that had colonised each panel were counted and recorded. The second set of panels was removed before completion of the experiment and no data are available on the numbers of ascidians recruited.

4.2.3 Asbestos panels (1992)

The ropes made up and deployed in 1990 (section 4.2.2) were reused. The cleaned asbestos plates were attached as previously using black cable-ties. The ropes with attached panels were suspended from the oil boom on May 10, 1992.

One set of panels was removed on August 2, 1992. The numbers of individuals of each of the three ascidian species of interest that had colonised each panel were counted and recorded. The second set of panels was removed on October 25, 1992. The numbers of individuals of each of the three species of interest that had colonised each panel were counted and recorded.

4.3 Results

4.3.1 Tufnol panels (1989)

After twelve weeks exposure the surface panel (Plate 21) was fouled with green algae (*Enteromorpha* sp., *Chaetomorpha* sp. and *Bryopsis plumosa*), colonial ascidians (*Botryllus schlosseri*, *B. leachi* and *Diplosoma listerianum*), barnacles, a few bryozoans (*Bugula stolonifera*) and hydroids (*Obelia* sp.). Green algae did not persist down to the 1 m depth panel; this was fouled with red algae (*Griffithsia flosculosa* and *Ceramium* sp.) and a brown alga (*Ectocarpus* sp.). The number of barnacles and area covered by colonial ascidians both increased. Some *A. aspersa* and a few *C. intestinalis* were present (Plate 22). *A. aspersa* exhibited intense recruitment at a depth of about 2 m (Plate 23) with some recruitment of *C. intestinalis*. Barnacles were abundant, but colonial ascidians and algae were rare. The 3 m depth panel was devoid of algae; barnacles were abundant but the numbers of *A. aspersa* and *C. intestinalis* declined (Plate 24). No *S. clava* were found on any of the panels. The numbers of the ascidians found are presented in Table 3.

TABLE 3 Distribution of solitary ascidians with depth after 12 weeks (1989)

Depth (m)	*A. aspersa*	*C. intestinalis*	*S. clava*
0	0	0	0
1	34	3	0
2	217	31	0
3	88	28	0

Plate 21 **Surface fouling community on tufnol panel after 12 weeks (1989)**

Plate 22 **1 m fouling community on tufnol panel after 12 weeks (1989)**

Plate 23 **2 m fouling community on tufnol panel after 12 weeks (1989)**

Plate 24 **3 m fouling community on tufnol panel after 12 weeks (1989)**

4.3.2 Asbestos panels (1990)

After twelve weeks exposure the surface panel was mainly fouled with green algae (*Enteromorpha* sp. and *Bryopsis plumosa*) and barnacles. One specimen of *A. aspersa* was found on this panel (Plate 25). At 1 m depth the panel was mainly fouled with red algae (*Griffithsia flosculosa* and *Ceramium* sp.), colonial ascidians (mainly *Botryllus schlosseri* and *Diplosoma listeriamum*), barnacles and a few *A. aspersa* (Plate 26).

Fouling by *A. aspersa* was intense on the 2 m depth panel (Plate 27) and considerably reduced on the panel from 3 m depth (Plate 28). *C. intestinalis*, barnacles, red algae (*G. flosculosa* and *Ceramium* sp.), brown alga (*Ectocarpus* sp.) and occasional colonial ascidians were also present on these deeper panels. No specimens of *S. clava* were found on any of the panels exposed in this experiment. The numbers of the ascidians found are presented in Table 4.

TABLE 4 Distribution of solitary ascidians with depth after 12 weeks (1990)

Depth (m)	*A. aspersa*	*C. intestinalis*	*S. clava*
0	1	0	0
1	19	2	0
2	739	59	0
3	238	33	0

Plate 25 **Surface fouling community on asbestos panel after 12 weeks (1990)**

Plate 26 **1 m fouling community on asbestos panel after 12 weeks (1990)**

Plate 27 **2 m fouling community on asbestos panel after 12 weeks (1990)**

Plate 28 **3 m fouling community on asbestos panel after 12 weeks (1990)**

4.3.3 Asbestos panels (1992)

After twelve weeks exposure the surface panel was fouled with green algae (*Enteromorpha* sp. and *Bryopsis plumosa*), red algae (*Griffithsia flosculosa* and *Ceramium* sp.), and barnacles (Plate 29). Three specimens of *A. aspersa* were present. The panel from 1 m depth was fouled with red algae (*G. flosculosa* and *Ceramium* sp.), barnacles, some patches of colonial ascidians (mainly *Botryllus schlosseri*), a few *A. aspersa* and one *C. intestinalis* (Plate 30). There were greater numbers of *A. aspersa* and *C. intestinalis* on the panel from 2 m depth (Plate 31) but numbers declined again on the panel from 3 m depth (Plate 32). Red algae (mainly *G. flosculosa*) and barnacles were frequent on these lower two panels. The numbers of the ascidians found are presented in Table 5.

Weed growth on the surface panel was less after 24 weeks exposure, the main fouling present being barnacles, very small *S. clava*, *A. aspersa* and *C. intestinalis* (Plate 33). The panel from 1 m depth was colonised by numerous small *S. clava* and *A. aspersa* (Plate 34). A few *C. intestinalis* were also present on this panel. The panel from 2 m depth supported a large population of *A. aspersa* with frequent *C. intestinalis*, occasional red algae (mainly *G. flosculosa*) but only one specimen of *S. clava* (Plate 35). Barnacles were present beneath the ascidians. The population of *A. aspersa* was reduced by almost half on the 3 m depth panel, but *C. intestinalis* numbers showed a smaller decline and the number of *S. clava* increased (Plate 36). Barnacles were abundant but macrophyte cover declined. The numbers of the ascidians found are presented in Table 6.

TABLE 5 Distribution of solitary ascidians with depth after 12 weeks (1992)

Depth (m)	*A. aspersa*	*C. intestinalis*	*S. clava*
0	3	0	0
1	26	1	0
2	188	34	0
3	80	11	0

TABLE 6 Distribution of solitary ascidians with depth after 24 weeks (1992)

Depth (m)	*A. aspersa*	*C. intestinalis*	*S. clava*
0	16	8	125
1	167	18	188
2	886	193	1
3	482	126	16

Plate 29 **Surface fouling community on asbestos panel after 12 weeks (1992)**

Plate 30 **1 m fouling community on asbestos panel after 12 weeks (1992)**

Plate 31 **2 m fouling community on asbestos panel after 12 weeks (1992)**

Plate 32 **3 m fouling community on asbestos panel after 12 weeks (1992)**

Plate 33 **Surface fouling community on asbestos panel after 24 weeks (1992)**

Plate 34 **1 m fouling community on asbestos panel after 24 weeks (1992)**

Plate 35 2 m fouling community on asbestos panel after 24 weeks (1992)

Plate 36 3 m fouling community on asbestos panel after 24 weeks (1992)

4.4 Conclusions

The three years of panel experiments indicate that for *A. aspersa* the greatest recruitment occurred at 2m depth after 12 weeks exposure commencing in May. *C. intestinalis* also showed a preferential, though much less intense, recruitment at this depth. No *S. clava* were found on any of the panels after twelve weeks exposure, suggesting that recruitment had either occurred so recently that the juveniles were too small to be seen with the unaided eye, or recruitment did not occur until after early August.

The panels exposed for 24 weeks in 1992 indicated that the distribution patterns for *A. aspersa* and *C. intestinalis* did not change over the additional time period. However, *S. clava* had settled in the intervening twelve weeks, with maximum recruitment occurring near the surface and a possible second population peak at 3 m depth.

The results of these field experiments indicate that there is initial recruitment zonation of the three solitary ascidian species, and the previous fortuitous observations are valid and repeatable. The questions that must now be addressed is "how is this recruitment zonation achieved?". Assuming, for the moment, that the zonation of juveniles is not the result of predation, then pre-settlement zonation of ascidian larvae in the water column would appear to be a necessary precursor to zonation of juvenile recruits. Laboratory experiments will be designed to identify the mechanisms by which pre-settlement larval zonation could be achieved.

CHAPTER 5 MAIN EXPERIMENTAL PROCEDURES

5.1 Larval culture

5.1.1 Adult stock maintenance

Adult ascidians were collected from the oil-boom mooring chains and other floating structures in the Fawley cooling water inlet during the low tide period. Specimens of *C. intestinalis* and *S. clava* were easily peeled from the substratum but, despite their thick tunic and tough appearance, specimens of *A. aspersa* were relatively fragile and easily damaged if separated too vigorously from the substratum. The organisms were returned to the laboratory and held in a moderate flow of aerated sand-filtered sea water, as recommended by Cloney (1987). All sea water used in this project was sand-filtered Fawley inlet water unless otherwise indicated. The holding tank was cleaned daily and dead specimens, faeces, silt and other debris were removed. Stock cleanliness is essential because under crowded conditions, with restricted water circulation, ascidians commonly become infested with a white microorganism (*Leucothrix* sp.) that spreads rapidly from specimen to specimen.

Prior to use as spawning stock, all epibionts were removed from the ascidians by gentle scraping and the ascidians sorted into species; any individuals that could not be thoroughly cleaned were discarded. It was noted that the only epibiont attached to *C. intestinalis* was *Botyillus schlosseri* (Pallas) which occurred infrequently. *B. schlosseri, Molgula manhattensis (De Kay)* and *Griffithsia flosculosa* Batters were frequently found on *A. aspersa*, together with an occasional commensal *Musculus marmoratus* (Forbes). *S. clava* supported many epibionts, including juvenile *S. clava*, *B. schlosseri* and *G. flosculosa.*

Holmes (1968) found that the culture of the larvae of *A. aspersa* and *S. clava* from artificial fertilisation was very difficult. Despite using gametes from apparently ripe individuals, he could not always obtain fertilised eggs and the larvae that did develop from the successful fertilisations were often abnormal in the tail region. As the objective of the present study was to carry out behavioural experiments on large cohorts of motile larvae of each ascidian species, it was considered that natural spawning of monospecific populations would be the most effective method of larval production (Rose, 1939). Therefore it was important to remove all epibionts from the spawning stock of solitary ascidians.

5.1.2 Induction of Spawning

Holmes (1968) found that fertilisation of the eggs of *A. aspersa* and *S. clava* was only possible between late June and early September. Therefore the majority of attempts to induce spawning for these species were carried out during this period.

As noted by Yamaguchi (1970), there was a tendency for *C. intestinalis* to spawn soon after collection and handling, even in conditions of constant light and temperature, but the yield of eggs was small and unreliable. The other two species did not spawn consistently as a result of this stimulus. In order to obtain a regular supply of eggs for experimentation it was necessary to develop a reliable technique to induce spawning.

Variation in light duration (i.e. period of light/dark alternation) and intensity can initiate spawning in *C. intsetinalis* (Berrill, 1947; Lambert & Brandt, 1967; Whittingham, 1967: Georges, 1971) and *Styela partita* (Rose, 1939). Initial attempts to induce spawning employed changes in the light/dark cycle at constant temperature. Batches of six cleaned ascidians were

kept in dishes of filtered (30 μm) sea water in a light/temperature-cycle incubator at 16°C with aeration. A variety of light/dark cycles were applied; exposure to 6-12 hours continuous light after 24 hours in the dark was the most effective regime tested. However, it was not always possible to obtain *C. intestinalis* eggs by this method and the yield was usually small and variable. Large plastic tanks were employed in an attempt to increase stock density and consequent egg yield, but without great success. Light was not found to be an effective spawning stimulus for *A. aspersa* or *S. clava*, although Rose (1939) used light to induce *Styela partita* to spawn. Indeed, as many specimens of *S. clava* were observed to be exposed at low water, light intensity might not be a reliable spawning stimulus for this species. In addition to the problems outlined above, it was considered possible that larvae hatched from eggs induced by light could be sensitised, particularly if part of the embryonic development occurred in the light, since the light levels used were higher than those normally encountered by the organisms; such sensitisation would confound experiments to test the response of the larvae to light. Therefore, a different spawning stimulus was sought.

The reproductive cycle in ascidians is closely linked with temperature (Berrill, 1975). Ambient water temperature appears to be an important factor in the initiation of spawning and, for *C. intestinalis* at least, a correlation has been proposed between spawning periodicity and seasonal temperature differences (Dybern, 1965). Sabbadin (1957) reported that *C. intestinalis* bred in the lagoons of Venice only when water temperatures exceeded 10-11°C, and Millar (1952) observed that this species spawned in the Clyde between July and September when, according to Barnes (1959), mean sea temperatures are usually between 13°C and 15°C. Holmes (1968) found temperature dependent breeding in *S. clava*; gonad development occurring when mean sea temperatures were higher than 8°C and gonad maturation when temperatures exceeded 16°C. Even in the warm waters of southern California, where minimum winter

temperatures of 14°C were recorded, Kelly (1974) observed *S. clava* breeding only in late summer when water temperatures ranged from 18°C to 22°C. Therefore temperature shock was considered a spawning stimulus that warranted further investigation.

The effect of temperature shock was investigated by adding heated filtered (30 μm) sea water to tanks containing cleaned ascidians in filtered (30 μm) sea water so as to rapidly increase the tank water temperature to a predetermined value. Yamaguchi (1970) noted that spawning induction by temperature shock was not always successful with *C. intestinalis* and Holmes (1972) reported that *S. clava* is not sensitive to rapid changes in temperature. But in preliminary experiments, increased egg yield was achieved by holding the spawning stock at the higher temperature for a few hours. So, once the temperature shock had been achieved, the tanks were placed in a water-bath at the target temperature for four hours, then allowed to cool to ambient temperature. A variety of final temperatures were tested; the best yields of eggs were achieved with target temperatures of 21-23 °C. A variety of light conditions were applied after the temperature shock but light was found to have little effect on egg yield. Consequently ascidians were allowed to spawn under subdued natural light conditions.

The standard method employed to induce spawning was as follows. Single species batches of up to 100 cleaned adults were placed in Netlon® baskets (0.2 m x 0.4 m, 0.2 m deep; 5 mm mesh) in plastic tanks (20 l) half-filled with filtered (30 μm) aerated sea water. Each basket was supported on a Netlon® plinth (5 mm mesh) which held it approximately 2 cm off the bottom of the tank. Hot filtered (30 μm) sea water was added to the tank to bring the water temperature to 21-23°C. The tank was then placed in a water-bath at approximately 22°C and the water jacket temperature maintained for four hours. The water-bath and tank were then allowed to cool

overnight to ambient temperature. Eggs were harvested the following morning, dead specimens replaced with fresh individuals and the process repeated. *A. aspersa* and *S. clava* often required several cycles of this procedure to induce spawning. Once the yield of eggs from a tank had reduced to a few hundred, the ascidians were completely replaced with fresh stock. The spawning facility used in this study is shown in Plate 37.

The Netlon® plinth permitted any negatively buoyant eggs produced to be held separate from the adults. Such eggs fell through the mesh of the basket and plinth and collected in the bottom of the tank where they were unavailable as a food source to the adult ascidians. Although cannibalism has not been recorded for these three ascidian species, such filter feeders may prey upon conspecific eggs and larvae since these are often not distinguished from other food (Thorson, 1950; Timko, 1979; Young & Gotelli, 1988). Indeed, Young (1988) has shown that some non-gregarious species of solitary ascidian consume conspecific eggs and larvae so it seemed a wise precaution to separate eggs and adults, particularly in view of the stock density in the spawning tank. The ascidian branchial basket filters all water passing through the animal, and the size of the branchial basket ostia sets the upper limit of the size of the particle that can pass through the filtering mechanism without being trapped. In *S. clava* the ostia measure about 300 µm by 60 µm yet individuals can retain food particles of about 4 µm (Holmes, 1968), so recently fertilised eggs could be retained and injested, particularly after they have undergone osmotic expansion (see Chapter 6). Similar ostia dimensions are be found in *A. aspersa* and *C. intestinalis*, but specimens of the former also retain food particles of about 4 µm (Holmes, 1968), and the latter have been shown to retain particles of about 1 µm diameter by trapping them in mucus produced by the endostyle (Jørgensen, 1949; Jørgensen & Goldberg, 1953); so the risk of egg retention, if not ingestion, is real. In addition, conspecific gametes have been found in the inhalent siphon of *C. intestinalis* specimens (Carlisle, 1951).

Plate 37 **Ascidian spawning tanks**

The great advantage of the procedure outlined above is that it permits large scale production of eggs leading to large numbers of larvae available for experimentation, providing a sounder basis for statistical inference. However, there is a caveat. It is possible that eggs spawned as a result of temperature shock may be subtly different in some way from eggs produced naturally, producing larvae that could be abnormal in physiology, morphology or behaviour. Such abnormal larvae would confound the results of experimentation.

5.1.3 Egg harvesting

Each morning the basket of ascidians was taken out of the tank, rinsed in filtered (30 μm) sea water to remove any trapped or adhered eggs spawned in the previous 24 hours, and transferred to another tank of fresh, filtered (30 μm) aerated sea water with a mesh plinth ready for further temperature shock treatment. The water in the used tank usually contained ascidian eggs and detritus. If eggs were incubated with detrital material, embryonic development could be affected, as is the case with *Crassostrea gigas* (Davis & Hidu, 1991); furthermore, particles of detritus often adhered to newly hatched larvae affecting their buoyancy. It was therefore considered important that the eggs should be separated from the detritus. Gentle filtration appeared the obvious technique; this also allowed the eggs to be concentrated, reducing the space and facilities necessary for incubation.

Sieves were made by gluing circles of 212 μm, 150 μm and 75 μm plankton netting onto one end of short lengths of 90 mm diameter ABS Durapipe®. The water that had contained ascidians overnight was sequentially filtered through the three sieves in order of decreasing mesh size. The 212 μm mesh acting as a pre-filter removing very large material, and the 150 μm mesh collected

the majority of large particle detritus and some eggs. Eggs and fine detritus were retained by the 75 μm mesh; increasing the finest mesh to 95 μm eliminated much of the fine detritus without loss of eggs. The residues in the 95 μm and 150 μm mesh sieves were washed several times with filtered (30 μm) sea water and the contents back-washed into crystallising dishes with filtered (10 μm) sea water. The residue from the 150 μm mesh sieve was examined for eggs and discarded unless substantial quantities had been retained. Residues containing eggs were transferred, with copious rinsing (10 μm filtered sea water), to beakers (600 ml) with aeration.

All glassware used in these experiments was pyrex and was cleaned by soaking in Decon®, rinsed thoroughly in tap water then left to soak overnight in filtered (10 μm) sea water. The necessity of using very clean apparatus was stressed by Morgan (1945) and Berrill (1947). Holmes (1968) attributed the high proportion of his *S. clava* larvae that hatched with tail abnormalities, such as tails that did not straighten out, to the use of contaminated glassware.

5.1.4 Hatching and harvesting larvae

Embryonic development is temperature dependent (*A. aspersa* - Knaben, 1952; *C. intestinalis* - Yamaguchi, 1970), so the hatching time of any eggs spawned during this temperature shock/decay treatment before ambient temperature was reached may bear little relation to the natural hatching time. Nevertheless, development should be normal. It was considered important, however, to avoid temperature shock when hatched larvae were later transferred to experimental apparatus, so it was necessary to ensure that hatching took place at ambient sea water temperature. Harvested eggs were kept in aerated filtered (10 μm) sea water in glass beakers (600 ml) which were kept in a water-bath of flowing sea water, i.e. at ambient sea water

temperature, and allowed to hatch naturally. The water-bath was kept in an area of low light intensity (50 lux maximum). Once harvested, the eggs were not disturbed further.

Preliminary experiments indicated reasonably consistent times of spawning and hatch, which dictated the times of experimentation. In order to obtain a supply of young larvae, it was merely necessary to observe the eggs regularly around the anticipated time of hatching. Unfortunately the eggs of the three species studied did not exhibit synchronised hatching so, although it was possible to sample larvae after one hour that were up to one hour old and so on, there was a tendency to generate a wider range of larval ages as hatching time progressed.

The possibility of net damage prohibited harvesting the larvae by filtration. Larvae tended to congregate at the surface, so they could be transferred to a dilution vessel or directly to the experimental apparatus by decanting an aliquot of surface water from the beaker (Cloney, 1987). The volume of the initial aliquot decanted was dependent on the density of larvae present, but was usually about 100 ml. Decanted water was replaced with fresh filtered (10 μm) sea water. Subsequent aliquot volumes increased as the concentration of larvae in the beaker decreased. This technique had several advantages. First, there was greatly reduced risk of damage resulting from handling larvae. Second, as larvae swam up shortly after hatching, decanting the surface layer continually provided a sample containing a high proportion of newly hatched larvae. Third, the larvae were separated from unhatched eggs and any detritus present which could adhere to them during experimentation and bias their behavioural responses; separation from detritus also facilitated subsequent counting. There are, of course, disadvantages inherent in this approach. The age range of larvae in each aliquot increased with the number of aliquots sampled, and it was impossible to use a constant number of larvae in each experiment; indeed, the actual number of larvae used in an experiment remained unknown until the preserved sample was counted.

5.2 Preservation and staining technique.

Formaldehyde was selected as the preservative on grounds of cost and availability. Rose Bengal and neutral red are commonly used to visualise micro-invertebrates; both were readily available so tests were carried out to identify the most appropriate stain. *C. intestinalis* larvae were killed with a 4% solution of formaldehyde and divided into two portions. One portion was stained with neutral red (0.05% w/v) plus glacial acetic acid (approximately 1 ml per 100 ml sample). The second sample was stained with Rose Bengal solution (1% in 40% formaldehyde) at a concentration of approximately 5%. The samples were stored for a week then re-examined.

Larvae stained with neutral red were very pale pink in colour and appeared to be surrounded by clouds of fine detritus which produced a blurred image. The larvae stained with Rose Bengal were dark red, clear and distinct. Detritus also took up Rose Bengal; nevertheless, the most suitable preservation/staining technique appeared to be Rose Bengal in formaldehyde solution.

5.3 Validation of filtering technique.

The first stage in determining the distribution of larvae resulting from experimentation was to remove the larvae from the formaldehyde solution. Filtration was the most appropriate technique; it had the added advantage of concentrating the larvae and separating them from detritus. But before it could be incorporated into the experimental protocol, it was essential that the efficacy of filtration was validated and the filtering technique standardised.

A series of simple sieves were made by gluing circles of plankton netting (30, 55 and 75 μm meshes) onto short lengths (approximately 80 mm) of 90 mm diameter ABS Durapipe®. Stained

preserved *C. intestinalis* larvae were filtered through the plankton netting filters and the filtrate was examined for any larvae that had passed through. The filters were then backwashed with filtered (10 μm) sea water. Initial washing was back and forth in a horizontal direction, then vertically. The washing procedure was carried out one, two and three times and the mesh examined to determine whether any larvae were retained. This was used as an indication of the relative efficiency of backwashing.

The filtrate from the 75 μm filter contained two larvae. No larvae were found in the filtrate from the other two filters; the 30 μm filter retained slightly more debris than the 55 μm filter but the amount was small. Backwashing for one cycle left five larvae on the filter. All larvae were removed from the filter when two and three cycles were employed.

The amount of material trapped on the 30 μm mesh was not considered a problem so this mesh was chosen to ensure all larvae were retained. The filtering method selected for preserved larvae was to pour the sample through 30 μm mesh plankton net supported on a section of 90 mm diameter ABS Durapipe®, rinse well and backwash for three cycles.

5.4 Counting method and efficiency.

Larvae were removed from the counting chamber as they were counted. The larvae were viewed and located with a binocular microscope, air was expelled from an Eppindorf pipette (200-1000 μl volume), the tip was introduced into the field of view and the larvae counted as they were sucked into the pipette tip. Batches of ten larvae were removed and recorded in order to reduce errors if counting was interrupted.

The counting routine was as follows. The dish was swirled to concentrate the larvae in the middle. The surface of the water was examined first and any larvae floating on the surface were counted and removed. Larvae settled around the bottom rim of the dish, and any larvae stuck to the side of the dish, were counted and removed. Larvae in the bottom of the dish were then counted and removed in horizontal passes across the dish. The dish was rotated through 90° and the horizontal passes across the dish repeated.

It was not possible to assess the counting efficiency by repeating the count because some larvae remained attached to the inside of the disposable pipette tip. Therefore the efficiency of the technique was determined by repeating the counting procedure two and three times to detect the presence of any previously uncounted larvae. All larvae were removed after two cycles, so a second counting cycle was incorporated into the standard procedure.

5.5 Construction and operation of the vertical behaviour chamber.

The vertical behaviour chamber was made from ¾ inch ABS class E Durapipe® pipe and fittings. Five Scorpion ¾ inch double union ball valves (plain socket, EPDM seals), aligned for flow upwards, were joined by four lengths of pipe (160 mm). A longer length of pipe (180 mm) was fitted to the top ball valve (Figure 9). The ball valves consisted of a 60 mm long chamber flanked by two 20 mm long sockets which received the pipe, so that each section of pipe and valve was 200 mm long; the water and larvae trapped in the closed valve were part of the sample held in the segment of the pipe below it. Plankton netting (30 μm mesh) was glued to the end of a short stub of ¾ inch pipe and the net end of the pipe was inserted into the bottom end of the bottom ball valve to prevent larvae escaping from the chamber. A hose fitting was glued onto the pipe stub and ¾ inch transparent hose (4 m) attached (Plate 38).

Figure 9 **The vertical behaviour chamber**

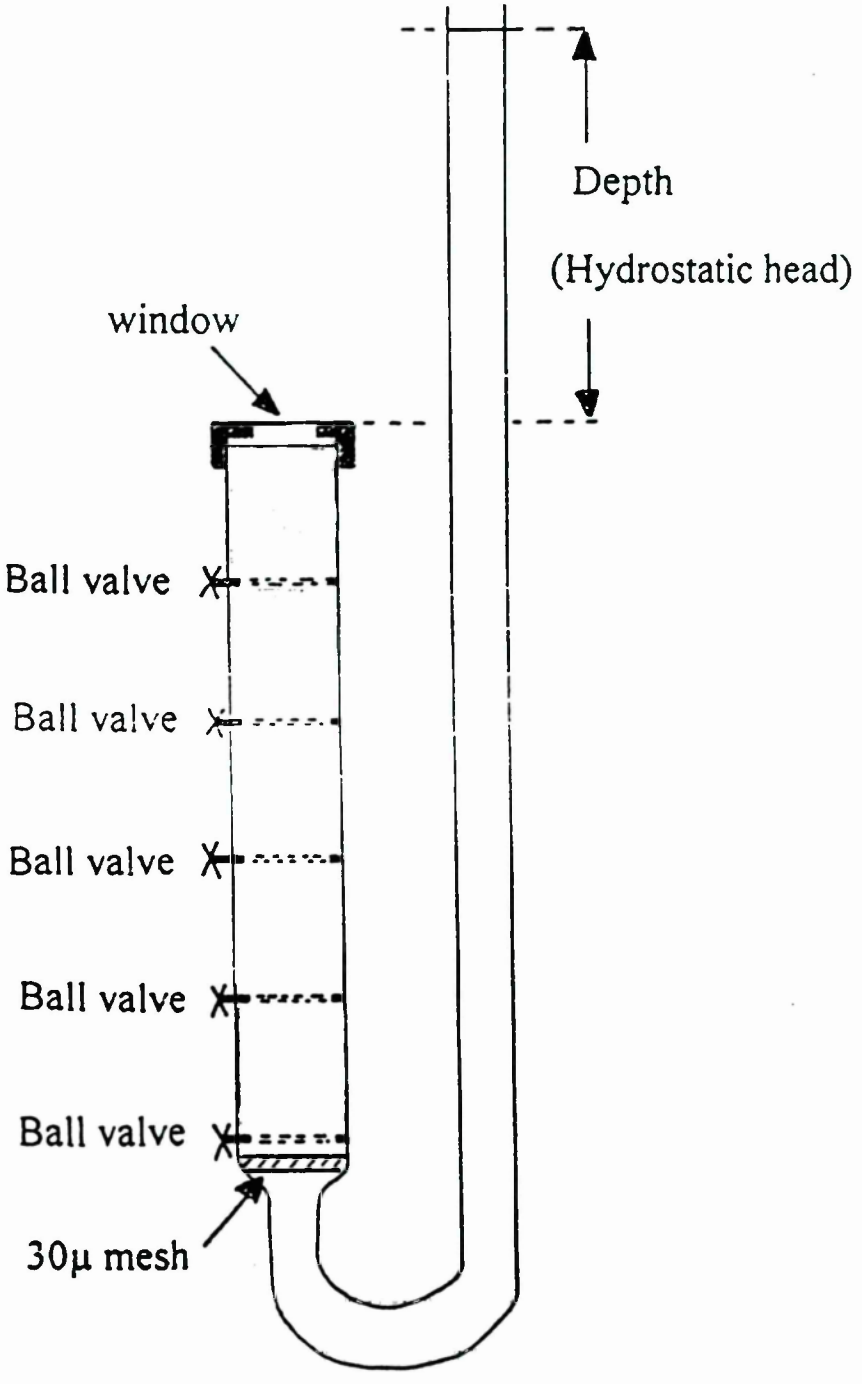


PLATE 38 **The vertical behaviour chamber**

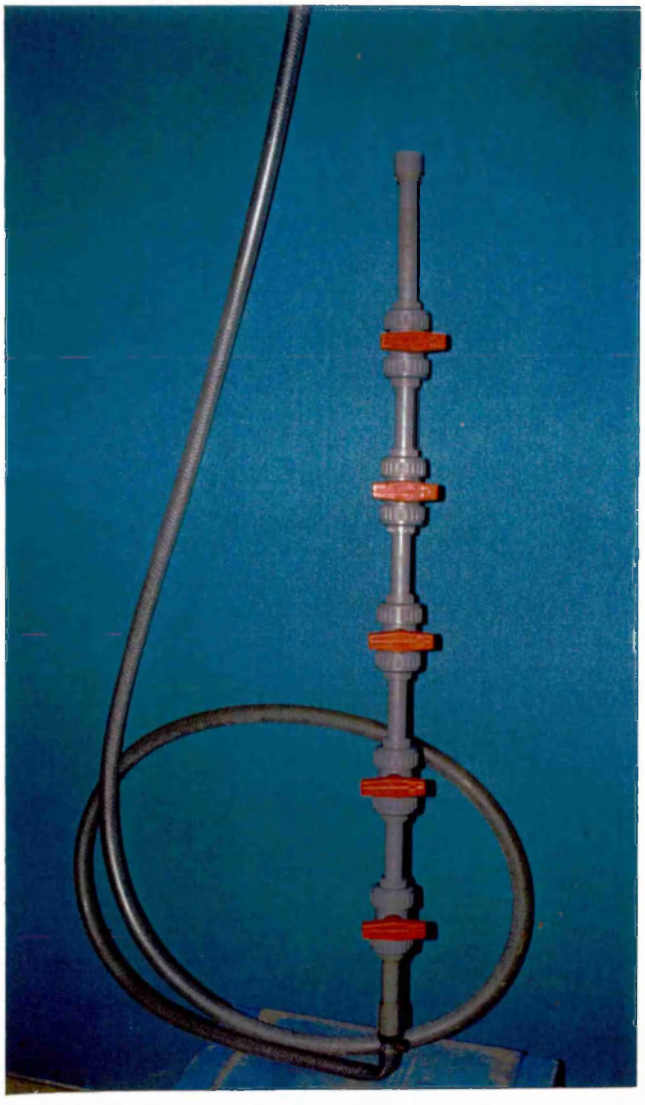
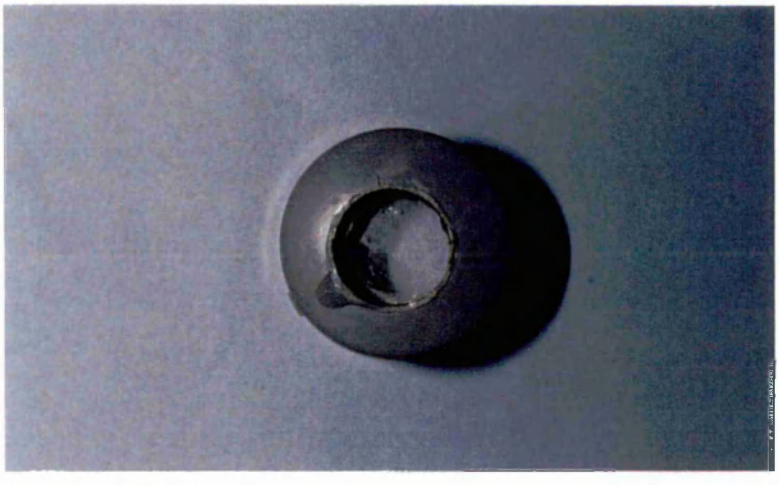


PLATE 39 **The window of the vertical behaviour chamber**



A pulley was attached to a roof beam above the experimental area. A rope was attached to the open end of the polythene tube, passed through the pulley and tied off at ground level. The hydrostatic pressure in the chamber could be varied by raising the end of the polythene tube. The rope was marked when the free end of the transparent tube was level with the top of the chamber, then the tube was raised and the rope marked at 0.5 m intervals of hydrostatic head.

A window fitting was constructed for the experiments in which larvae were to be exposed to light. This was made by drilling a ¾ inch hole in a one inch ABS end cap and inserting a one inch glass circle into the end cap; the glass circle was held firmly in place by gluing a ¾ inch ABS adapter inside the end cap to produce a glass sandwich (Plate 39). For those experiments without light, a rubber bung was inserted firmly into the top of the tube.

The operating procedure for the vertical behaviour chamber was as follows. All valves of the behaviour chamber were opened and the complete system was filled with filtered (10 μm) sea water. The valve connecting the variable head tube and the behaviour chamber was closed and the chamber drained. Ascidian larvae were decanted into filtered (10 μm) sea water (350 ml) and the diluted ascidian larvae immediately transferred to the behaviour chamber. The chamber was filled to overflowing and the window was fitted to, or the rubber bung inserted in, the top of the tube. The chamber was inverted five times to ensure thorough mixing, then fixed in the vertical plane. The bottom valve was opened and the hydrostatic head adjusted by raising the open end of the polythene tube. The chamber was left at constant temperature for one hour. Where appropriate, the light level was checked every few minutes and adjusted as necessary. At the end of this time period all valves were closed to isolate the segments of the chamber. Each segment of water was decanted in turn, with rinsing, into labeled sample pots. The samples were stained and preserved (section 5.2) for later examination.

A series of preliminary experiments were carried out to determine the optimum duration of the experiment. Larval distribution in the absence of light was reasonably constant after one hour so this was adopted as the standard experimental period.

Two sets of control experiments were carried out. In the first set the larvae were introduced into the tube as usual, the tube was inverted five times then the segments of water drained immediately into sample pots. This control was designed to determine the initial distribution of the larvae. The second set of controls used larvae which had been anaesthetised with a solution of ethyl p-aminobenzoate in ethanol (approximately 0.1%); these larvae were treated just as experimental larvae. This control was designed to determine the passive movement of the larvae due to convection currents, vibration of apparatus etc.. These experiments were also used to determine the buoyancy of the larvae (Chapter 7).

The results of the vertical behaviour chamber experiments are reported using the following convention. The number (and %) of larvae in the chamber sections at the end of each experiment are tabulated vertically. Each column represents an experiment, with the first entry in the column representing the number (and %) of the larvae in the top section of the chamber, the second entry representing the number (and %) of the larvae in the second section of the chamber and so on. The last entry in the column is the total number of larvae used in the experiment. The results of the initial distribution control experiments are presented in Tables 7, 8 and 9 as examples. The distributions of *C. intestinalis* and *A. aspersa* did not differ significantly ($p<0.01$, χ^2-test) from random; although similar proportions of *S. clava* larvae were found in each section of the vertical behaviour chamber, the distributions of these larvae differed significantly ($p>0.01$, χ^2-test) from random.

Some distributions are presented graphically as vertical bar charts composed of five segments. The top segment represents the % of larvae found in the top section of the vertical behaviour chamber, the second represents the % of the larvae in the second section of the chamber and so forth (Figure 10).

Table 7 **Initial distribution (and %) of *C. intestinalis* larvae in the vertical behaviour chamber (active control)**

	Control Expt.1	Control Expt.2	Control Expt.3	Mean active control
Section A (top)	197 (21.8)	195 (18.8)	139 (22.5)	177 (20.8)
Section B	155 (17.2)	198 (19.1)	97 (15.7)	150 (17.6)
Section C	158 (17.5)	166 (15.0)	121 (19.6)	148 (17.4)
Section D	206 (22.8)	262 (25.2)	119 (19.3)	196 (22.9)
Section E (bottom)	187 (20.7)	218 (21.0)	141 (22.9)	182 (21.3)
Total number of larvae	903	1039	617	853

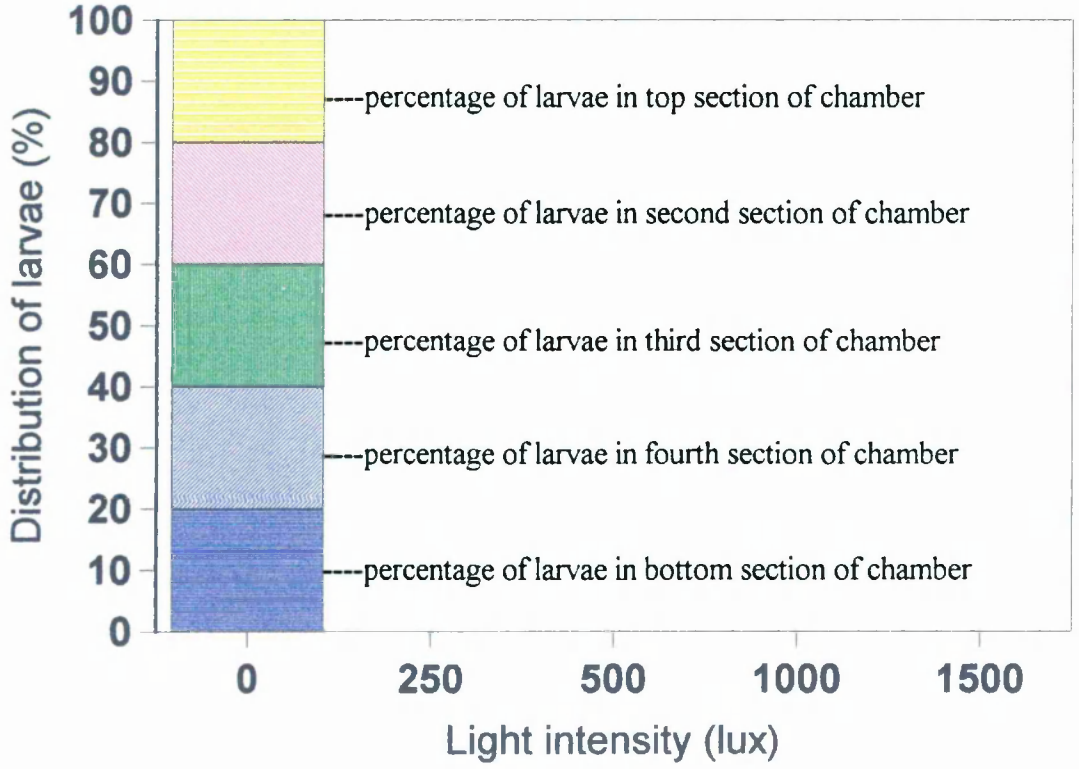
Table 8 **Initial distribution (and %) of *A. aspersa* larvae in the vertical behaviour chamber (active control)**

	Control Expt.1	Control Expt.2	Control Expt.3	Mean active control
Section A (top)	57 (17.8)	114 (17.2)	288 (18.5)	153 (18.1)
Section B	75 (23.4)	140 (21.1)	366 (23.5)	194 (22.9)
Section C	72 (22.5)	163 (24.6)	358 (23.0)	198 (23.4)
Section D	55 (17.2)	124 (18.7)	263 (16.9)	147 (17.4)
Section E (bottom)	61 (19.1)	121 (18.3)	280 (18.0)	154 (18.2)
Total number of larvae	320	662	1555	846

Table 9 **Initial distribution (and %) of *S. clava* larvae in the vertical behaviour chamber (active control)**

	Control Expt.1	Control Expt.2	Control Expt.3	Mean active control
Section A (top)	288 (17.6)	791 (15.4)	389 (16.8)	489 (16.1)
Section B	359 (22.0)	1230 (23.9)	536 (23.2)	708 (23.4)
Section C	364 (22.3)	1170 (22.7)	502 (21.7)	679 (22.4)
Section D	315 (19.3)	953 (18.5)	430 (18.6)	566 (18.7)
Section E (bottom)	309 (18.9)	1001 (19.5)	455 (19.7)	588 (19.4)
Total number of larvae	1635	5145	2312	3031

Figure 10 **Convention for representing the distribution of larvae in the vertical behaviour chamber.**



5.6 Construction and operation of the horizontal behaviour chamber.

The body of the chamber was made from a 1 m length of 90 mm diameter PVC Durapipe®. The pipe was cut in half lengthwise and four semi-circular baffles (3 mm PVC sheet) were glued into one half at 20 cm intervals. A hole (13 mm) was drilled in the base of this half pipe approximately 1 cm from the designated front end of the pipe, and another just behind of each baffle. A short stub of pipe (approximately 20 mm x 13 mm diameter) was glued externally into each hole so as to leave no internal ridges. A hole (6 mm) was drilled in the base of the other half pipe approximately 18 cm from the front end, then four more at 20 cm intervals. The two halves of the pipe were then glued together to reform the tube (Figure 11).

Two holes (13 mm) were drilled in a 100 mm square of PVC sheet (3 mm thickness), each approximately 1 cm in from opposite edges. This square was glued onto the back end of the tube such that the holes lined up with the holes in the tube (Figure 11). Two 100 mm and two 90 mm strips of perspex sheet (5 mm wide x 3 mm thick) were glued edge-on and sandwiched between two 100 mm squares of perspex sheet to form a narrow box. Two holes (3 mm) were drilled in opposite ends of one of the strips and polythene nipples glued in to permit circulation of cooling water. Three further strips and another sheet of perspex were glued onto the periphery of one face of the box to form a filter holder. This unit was then glued onto the front end of the tube (Figure 11).

A dexion® frame was constructed to support the tube. Four wheels were attached to the inside of the frame at the points of contact with the tube, so as to allow the tube to rotate freely in the frame (Plate 40).

Figure 11 **The horizontal behaviour chamber**

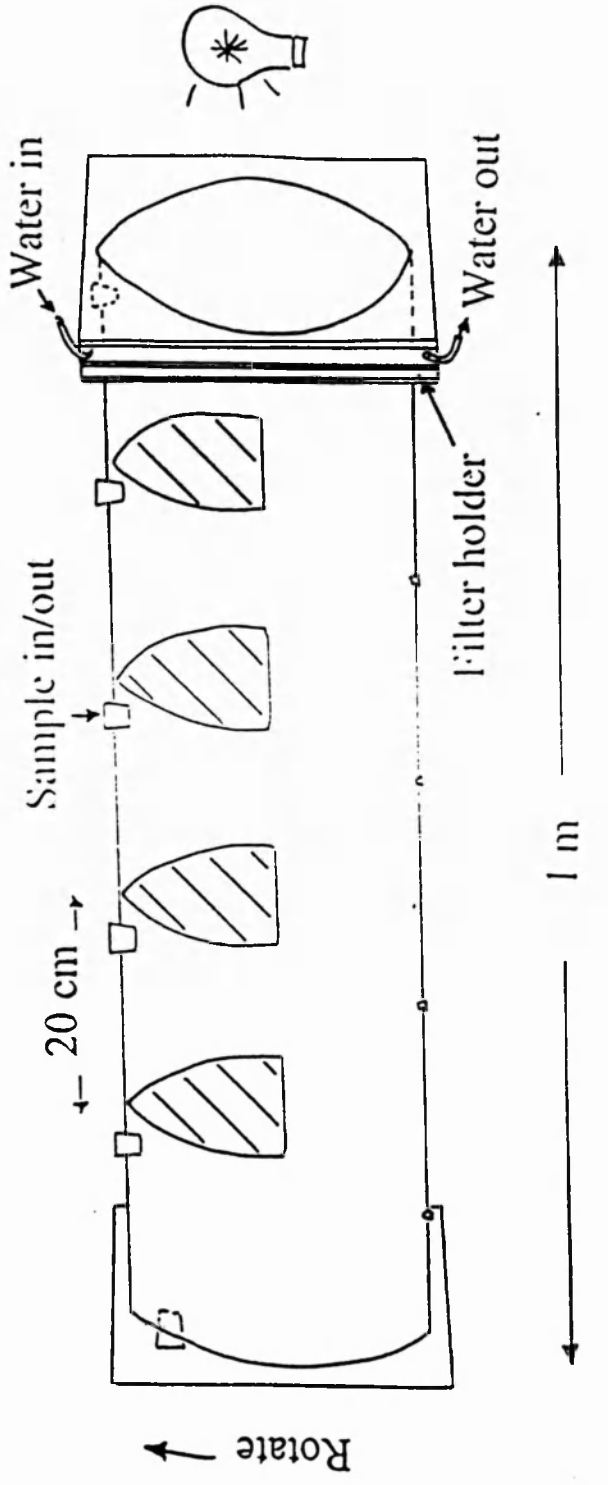


Plate 40 **Filling the horizontal behaviour chamber**

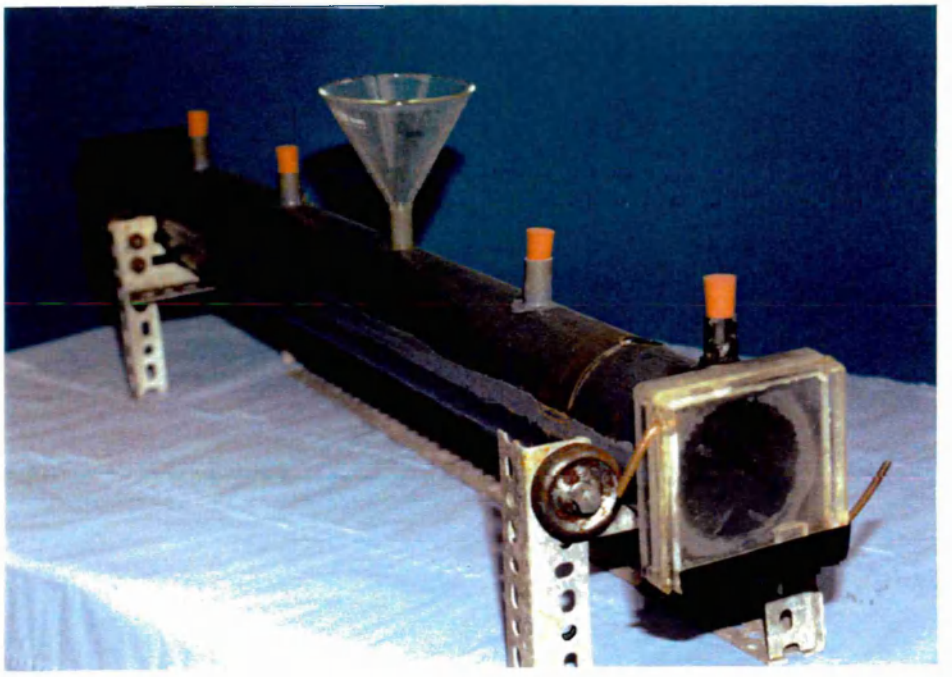
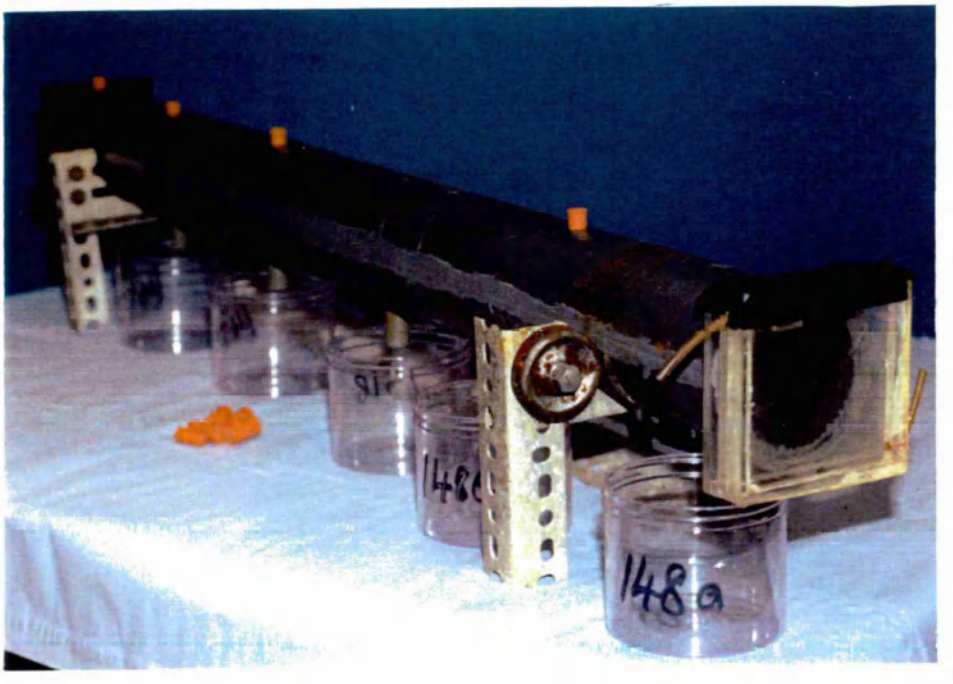


Plate 41 **Emptying the horizontal behaviour chamber**



The typical experimental procedure was as follows. Rubber bungs were inserted into the 6 mm holes and the tube rotated so that they were at the bottom (Plate 40). Where appropriate, the tube was aligned with the light source by viewing through the bottom hole of the end plate. Both end-plate holes were then sealed with rubber bungs. Filtered (10 μm) aerated sea water (approximately 1900 ml) was poured into the chamber via the middle port and allowed to stand for a few minutes to become quiescent.

An aliquot (approximately 100 ml) of water and larvae was decanted slowly from the hatching beaker into the tube through the middle entry port, using a glass funnel with the chamfered edge parallel to the side of the tube so as to avoid directing the momentum of the larvae along the axis of the tube (Plate 40). Rubber bungs were then inserted into the holes to exclude light. The tube was left for one hour at constant temperature during which time, where appropriate, the light level was checked every few minutes and adjusted as necessary. The tube was then rotated to isolate the segments of liquid. The (now) top bungs were removed to allow air ingress and the segments of water drained into sample pots (Plate 41). The segments of tube were rinsed (10 μm filtered sea water) through the top holes, and the samples preserved (section 5.2).

A series of experiments were carried out to find the optimum experimental time and larval insertion port. Experiments in which larvae were introduced via the middle port showed negligible change in larval distribution after one, two and three hours duration. Therefore one hour was selected as the standard experimental exposure time. The insertion port used affected the subsequent distribution of larvae so it was decided to use the middle port to characterize the magnitude and direction of movement, and other ports to examine movement in more detail.

Two sets of control experiments were carried out. In the first set the larvae were introduced into the tube as usual, but the tube was rotated immediately and the segments of water drained into sample pots and preserved. This control was designed to determine the initial distribution of the larvae resulting from the momentum associated with the introduction of the slug of water. The second set of controls used preserved, stained *C. intestinalis* larvae from recent experiments. These larvae were treated just as experimental larvae. This control was designed to determine the passive movement of the larvae due to convection currents, vibration of apparatus etc.. Anaesthetised larvae were not used for this control because there was a possibility that they could partially recover unless the receiving water was also treated, which substantially increased experimental costs. It was assumed that all dead larvae would behave similarly.

The results of the horizontal chamber experiments are reported using the following convention. The number (and %) of larvae in the chamber sections at the end of each experiment are tabulated horizontally. Each row represents an experiment, with the first (left hand) entry in the row representing the number (and %) of the larvae in the section of the behaviour chamber closest to the transparent end (light source for light experiments); the second entry in the row representing the number (and %) of the larvae in the second section of the chamber and so on across to the penultimate entry, which represents the number (and %) of the larvae in the section of the behaviour chamber furthest from the transparent end. The last entry in the row is the total number of larvae used in the experiment. The results of the initial distribution control experiments are presented in Tables 10, 11 and 12 as examples. The results of the control experiments with dead larvae are presented in Tables 13. All distributions differed significantly ($p>0.01$, χ^2-test) from random, with the largest proportion of larvae found in the section of the horizontal chamber closest to the entry port.

Table 10 **Initial distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber (live control)**

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Expt. 1	0 (0)	435 (39.2)	658 (59.3)	16 (1.4)	0 (0)	1109
Expt. 2	2 (0.8)	93 (35.4)	146 (55.5)	22 (8.4)	0 (0)	263
Expt. 3	1 (0.1)	297 (35.9)	464 (56.1)	65 (7.9)	0 (0)	827
Mean live control	1 (0.1)	275 (37.5)	422.7 (57.7)	34 (4.7)	0 (0)	733

Table 11 **Initial distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber (live control)**

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Expt. 1	0 (0)	163 (40.1)	239 (58.9)	4 (1.0)	0 (0)	406
Expt. 2	5 (0.8)	242 (36.5)	381 (57.5)	32 (4.8)	3 (0.5)	663
Expt. 3	3 (0.5)	217 (37.2)	342 (58.6)	21 (3.6)	1 (0)	584
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551

Table 12 **Initial distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber (live control)**

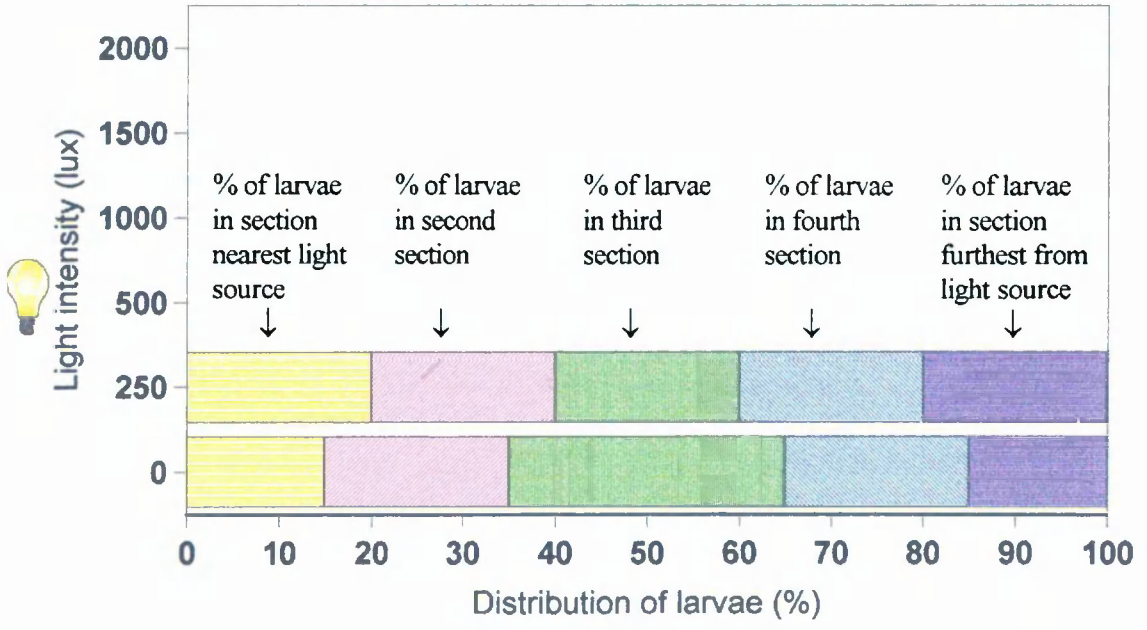
	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Expt. 1	0 (0)	303 (39.7)	416 (54.5)	45 (5.9)	0 (0)	764
Expt. 2	4 (0.5)	145 (39.1)	199 (53.6)	24 (6.5)	1 (0.3)	373
Expt. 3	3 (0.4)	296 (36.4)	455 (56.0)	58 (7.1)	1 (0.1)	813
Mean live control	2 (0.4)	248 (38.2)	357 (54.9)	42.3 (6.5)	0 (0)	650

Table 13 **Initial distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber (dead control)**

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Expt. 1	35 (3.1)	425 (37.6)	508 (45.0)	152 (13.5)	10 (0.9)	1130
Expt. 2	31 (1.3)	668 (27.1)	1132 (54.0)	374 (15.2)	62 (2.5)	2467
Expt. 3	35 (2.0)	603 (33.2)	878 (48.3)	252 (13.9)	48 (2.6)	1816
Mean live control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804

Some distributions are presented graphically as horizontal bar charts composed of five segments. The left hand segment represents the % of larvae found in the section of the horizontal behaviour chamber closest to the transparent window, the second segment represents the % of larvae found in the second section of the chamber and so forth (Figure 12).

Figure 12 **Convention for representing the distribution of larvae in the horizontal behaviour chamber**



5.7 Statistical analysis

Larval distributions were compared by testing the goodness of fit of frequencies arranged in a one-way classification using the *G*-test (Zar, 1984), with four degrees of freedom. To test larval response to a cue or combination of cues within each group of experiments, the mean proportions found in the end sections of the behaviour chamber with each set of experimental parameters were compared (after arcsine transformation) using the *t*-test (Zar, 1984). The effects of light and hydrostatic pressure on the distribution of larvae were estimated by carrying out a two way analysis of variance (Zar, 1984) on the proportions of larvae (after arcsine transformation) found in a particular section of the behaviour chamber.

A chi-squared test (Zar, 1984) was used to test for the random distribution of larvae in field samples collected through the water column (Chapter 14).

The experiments were carried out over six years and took pace in no particular order. They were opportunistic rather than randomised because larval availability was restricted to particular periods during the summer months. Natural light was used for the majority of the experiments (see Chapter 10 for the only exceptions), so the particular experiment carried out depended both upon the availability of larvae and the light conditions at the time. Dark experiments were carried out when light conditions were unfavourable.

CHAPTER 6 DIEL PATTERNS OF SPAWNING AND HATCHING

6.1 Introduction

Many organisms show diel patterns of larval release, with sunrise and sunset frequently controlling release (Branford, 1978). Light is known to trigger larval release in colonial ascidians (Millar, 1971), suggesting that larvae are released in the field shortly after dawn. Diel release time can influence initial survival of larvae through differences in predator activity (Hobson & Chess, 1978); visual predators may be avoided by night-time release and some corals release larvae only at night (Richmond & Jokiel, 1984) but others have maximum larval release at low tide, regardless of time of day (Holloran & Witteman, 1986), indicating that state of tide can also act as a cue.

For organisms with short-lived larvae such as colonial ascidians, time of release closely corresponds to settlement time and therefore determines the settlement cues available. Most colonial ascidians examined have been observed to release larvae only during the day (Olson, 1983; Svane & Young, 1989); for these larvae, light is likely to be an important factor in selecting suitable locations for settlement. For oviparous solitary ascidians the diel hatching time of larvae will determine the environmental cues available to larvae (e.g. light, tidal height, salinity). Combined with knowledge of the length of the larval phase, time of hatch may provide a clue as to what cues are likely to be important for settlement. If hatching occurs during the hours of darkness, phototaxis is unlikely to be involved in determining the position of the young larvae in the water column and, depending upon the life-span of the larvae, it may be an unlikely settlement cue; however, if hatching occurs in daylight, phototaxis could play an important role in pre-settlement behaviour.

The time period between spawning and hatching (i.e. the embryonic development period) can provide an indication of the passive dispersive potential of the ascidian species and consequently its ability to colonise new areas. Combined with knowledge of the sinking rate and buoyancy of the eggs, the embryonic development period may also provide a clue to the depth at which hatching occurs and thus the hydrostatic pressure and light level to which the newly hatched larvae are initially exposed.

For organisms whose larvae usually settle within minutes or hours of release, such as colonial ascidians (Millar, 1971; Duyl *et al.*, 1981; Olson, 1983; Stoner, 1989; Davis & Butler, 1989), variation in larval release may determine both larval availability and the timing of settlement. The number available for settlement is obviously determined by the number of larvae released but, by controlling release times, adults of species with short-lived larvae may influence larval supply as well as the actual timing of settlement.

Larval behavioural patterns that influence the location of settlement sites may themselves be affected by the diel timing of settlement because many of the environmental cues available to settling larvae vary with time of day. This is particularly true of light intensity. Larvae of many species are responsive to light level at the time of settlement in both the laboratory (Crisp & Ghobashy, 1971; Meadows & Cambell, 1972; Miller & Hadfield, 1986) and the field (Olson, 1983). If hatching occurs at a specific time period after spawning, the time of spawning could be instrumental in determining which environmental cues can play a role in settlement. In this situation light will only be a reliable cue for larvae if spawning is geared to the day-night rhythm; if it is geared to the tidal cycle, it will progress through the day until hatching eventually occurs in the dark. Thus the relationship of spawning and hatching with state of tide and dawn/dusk should be examined if light is to be proposed as a major cue.

6.2 Methods

6.2.1 Spawning time

Temperature shock was used to induce spawning within the time-scale of the experiment; it was assumed that this would have little effect upon the actual time of spawning or hatching. A batch of approximately 50 adults was given a temperature shock (1 hour at 22°C) then maintained in filtered (30 µm) sea water in a netlon® basket supported on a plinth (section 5.1.2). The temperature of the receiving water was noted. The basket was transferred to a fresh tank of filtered (30 µm) sea water with plinth every hour for up to 22 hours. Every effort was made to minimise the disturbance to the adults during transfer. The water in the initial tank was filtered and back-washed into a sample jar, stained and preserved for later examination (section 5.2). The tank was cleaned and refilled with filtered sea water ready to receive the next transfer of the ascidians. The time of first appearance of eggs was determined by examination of the samples. The experiment was repeated at various times during the spring-neap tidal cycle for each species.

6.2.2 Hatching time

Preliminary observations indicated that eggs of *C. intestinalis* hatched before 0700h, eggs of *A. aspersa* hatched between 1200 and 1600h, and those of *S. clava* hatched between 1000 and 1400h. To obtain a more precise estimate, twenty-two jars were half filled with filtered (30 µm) sea water, aeration introduced and eggs from a large spawning of each species (contemporaneous with the spawning experiment) were shared between the jars. Every hour the contents of one jar was stained and preserved for later examination. The time of first appearance of larvae was determined by examination of the preserved samples.

6.3 Results

6.3.1 *Ciona intestinalis*

On September 8, 1990, the experiment ran for 14 hours and the maximum spawning occurred between 1200h and 1300h (Table 14); receiving water temperature was 18.6°C. The experiment took place at extreme spring tide with a tidal range of 4.4 m and high water at 1343h BST; sun rise occurred at 0625h BST and sunset at 1929h BST. The first larvae appeared at 2330h the same day. On September 11, 1993, eggs appeared throughout the longer (22 hour) experiment, but the greatest spawning rate occurred at approximately 1400 h (Table 15). Water temperature was 20.2°C. This experiment took place at extreme neap tide, the tidal range was 1.5 m and high water was at 0712h BST; sun rise occurred at 0630h BST and sunset at 1915h BST. The first larvae appeared at 2200h the same day.

The time of hatching appeared to be independent of state of tide but was constant with respect to dawn/dusk. It was not clear if the time of spawning was independent of these cues. Larvae were generally present by 0700h and did not usually show indications of metamorphosing until 1900h the same day. On the occasions when hatching was observed, the process was complete within a few minutes and was followed by a period of approximately ten to fifteen minutes in which the larvae "twitched" in circles on the bottom of the beaker before they began to swim up towards the surface. Observations of hatch time suggest that the period of embryonic development is dependent on water temperature, as reported by Berrill (1935). The exceptionally warm conditions encountered in the summer of 1995 appeared to decrease this lag time but larvae were still in the water column during daylight hours.

TABLE 14 Spawning time of *Ciona intestinalis* (8/9/90)

Time (h, BST)	Number of eggs
1000	Animals installed in first tank
1100	6
1200	409
1300	744
1400	83
1500	16
1600	0
1700	8
1800	12
1900	0
2000	3
2100	0
2200	0
2300	0
2400	0

Water temperature = 18.6°C

TABLE 15 Spawning time of *Ciona intestinalis* (11/9/93)

Time (h, BST)	Number of eggs
2100	Animals installed in first tank
2200	11
2300	13
2400	10
0100	10
0200	4
0300	117
0400	16
0500	0
0600	48
0700	3
0800	27
0900	14
1000	3
1100	8
1200	58
1300	84
1400	352
1500	66
1600	21
1700	2
1800	0
1900	0

Water temperature = 20.2°C

6.3.2 *Ascidiella aspersa*

On August 10, 1991, spawning occurred in the evening between 1600h and 1900h with water temperature of 18.9°C (Table 16). The experiment took place the day before extreme spring tide. The tidal range was 4.2 m and high water was at 1148h BST; sun rise occurred at 0537h BST and sunset at 2034h BST. The first larvae appeared at 1400h the following day.

On August 27, 1993, spawning was less discrete, spanning 1400h-2000h (Table 17) but the first larvae appeared at 1300h the following day, approximately the same time as in the previous experiment. The receiving water initial temperature was 21.6°C. The experiment took place at extreme neap tide. The tidal range was 2.1 m and high water was at 0819h BST; sun rise occurred at 0605h BST and sunset at 1951h BST.

The experiments took place at the end of the *A. aspersa* reproductive season. Temperature shock was used to induce spawning and in 1993 the receiving water was initially at shock temperature. Nevertheless these results agree well with the observations of Knaben (1952), who reported an 18 hour lag to hatching at 20°C which reduced to 16 hours at 22°C, and Holmes (1968), who reported that hatching occurred about 18 to 20 hours after fertilisation at 20°C. The few opportunistic observations of hatching suggested that *A. aspersa* larvae required slightly longer to hatch than *C. intestinalis* larvae, but swim up occurred sooner.

Larvae were generally present in the hatching beakers by mid-afternoon. On the limited data collected, the time of spawning and hatching appears to be independent of state of tide but constant with respect to dawn/dusk.

TABLE 16 Spawning time of *Ascidiella aspersa* (10/8/91)

Time (h, BST)	Number of eggs
0800	Animals installed in first tank
0900	0
1000	0
1100	0
1200	0
1300	0
1400	0
1500	0
1600	56
1700	253
1800	366
1900	292
2000	0
2100	0
2200	0
2300	0
2400	84

Water temperature = 18.9°C

TABLE 17 Spawning time of *Ascidiella aspersa* (27/8/93)

Time (h, BST)	Number of eggs
1000	Animals installed in first tank
1100	0
1200	0
1300	0
1400	29
1500	4
1600	112
1700	281
1800	207
1900	311
2000	33
2100	0
2200	0
2300	0
2400	0

Water temperature = 21.6°C

6.3.3 *Styela clava*

On September 13, 1992, spawning occurred in the evening between 1800h and 2200h and the first larvae appeared at 0930h the following day (Table 18). The receiving water initial temperature was 16.8°C. The experiment coincided with extreme spring tide, with a tidal range of 3.9 m and high water at 1233h BST; sun rise occurred at 0635h BST and sunset at 1915h BST. On August 28, 1993, maximum spawning rate occurred between 2000h and 2400h and the first larvae appeared at 1200h (Table 19). The receiving water temperature was 21.5°C. The experiment took place the day after extreme neap tide, the tidal range was 2.4 m and high water was at 0932h BST; sun rise occurred at 0600h BST and sunset at 2000h BST.

These embryonic development periods agree well with Holmes (1968), who reported that hatching occurred 10-15 hours after fertilisation over the temperature range 16-23°C, and Na & Lee (1977), who reported that hatching occurred 14 hours after fertilisation at 19°C. Hatching took between five and ten minutes. The larvae began to swim up within a few minutes of hatching, and tended to form a column in the centre of the beaker; some larvae rose and sank, but many appeared to hold station in the water. Formation of the column of larvae was more pronounced if the beaker was placed in a well lit situation.

On the limited data available, it would appear that the times of spawning and hatching are independent of state of tide but are constant with respect to dawn/dusk. Hatch time appeared to vary with temperature; larvae were usually present by midday, generally well before when water temperature was in the range 18-20°C and occasionally after midday when the hatching water temperature was in the range 14-16°C.

TABLE 18 Spawning time of *Styela clava* (13/9/92)

Time (h, BST)	Number of eggs
0900	Animals installed in first tank
1000	0
1100	0
1200	0
1300	0
1400	0
1500	0
1600	0
1700	0
1800	54
1900	634
2000	266
2100	27
2200	0
2300	0
2400	0
0100	0

Water temperature = 16.8°C

TABLE 19 Spawning time of *Styela clava* (28/8/93)

Time (h, BST)	Number of eggs
0800	Animals installed in first tank
0900	0
1000	0
1100	0
1200	0
1300	0
1400	0
1500	0
1600	0
1700	0
1800	0
1900	0
2000	68
2100	112
2200	36
2300	88
2400	6
0100	0
0200	0
0300	0
0400	0
0500	0

Water temperature = 21.5°C

6.4 Discussion

Berrill (1947) noted that *C. intestinalis* normally spawns at dawn, but spawning can be induced at any time in the laboratory by short exposure to light following a period of darkness (Costello *et al.*, 1957; Lambert & Brandt,1967; Whittingham,1967) Light is an important spawning cue for *C. intestinalis* because spawning is controlled by a photoreceptor, located at the top of the spermatoduct (Reese,1967). Indeed in their review of ascidian ecology, Svane and Young (1989) point out that all solitary ascidians that have been reported to spawn early in the morning have transparent or translucent tunics. However, Yamaguchi (1975) reported that *C. intestinalis* spawned after dark in Japan, and Svane & Haverhand (1993) noted that field populations of *C. intestinalis* could spawn and settle at any time of day. In the present experiments, although some eggs were shed at daybreak, most eggs were found in the early afternoon. The observed time of spawning may not be representative of the natural time of spawning because the adults were subjected to handling, temperature shock and periodic movement which may have affected spawning. Temperature shock was considered necessary to ensure that spawning occurred within the observation period.

Movement from an area of one light intensity to another may also have affected the time of spawning. In the first experiment with *C. intestinalis* (8/9/90) the adults were moved from dim light to slightly brighter diffused light, which may have triggered spawning.

The total number of eggs produced was quite small and it is possible that induction took place before the adults were fully ripe. Yamaguchi (1975) reported that *C. intestinalis* spawned in approximately three day cycles in which gametes were shed into the gonoduct

for several successive days then spawned when the oviduct was full. In the present study large numbers of eggs were produced 1,4 and 9 days after induction with most batches.

Svane and Young (1989) suggest that not only do ascidians with transparent tunics spawn in the early morning, but that the reverse of this relationship may also be true. They noted that every solitary stolidobranch species with an opaque tunic that had been examined spawned many hours after the onset of light stimulation, e.g. *Styela plicata* (Yamaguchi, 1975: West & Lambert, 1976) and *Halocynthia roretzi* (Hirai & Tsubata, 1956). In the present study *A. aspersa* spawned in the late afternoon and *S. clava*, which has a thicker and less transparent tunic, spawned in the early evening; the related species *S. partita* has been observed to spawn between 1600h and 1900h (Rose, 1939). Insufficient experiments were carried out to determine whether the difference in spawning time between *A. aspersa* and *S. clava* was significant.

Although time of spawning could have been affected by the temperature shock applied, the animals were in water at ambient temperature by the time the eggs were fertilised so the lag times and time of hatch should be a reasonable estimate of the natural times. *C. intestinalis* larvae hatched just before midnight on the day of spawning at water temperatures of 14-18°C. Some larvae were still hatching 12 hours later and the larvae remained active until at least 2000h on the day after spawning, when many settlement stage larvae were observed. Thus larvae of this species were active throughout the daylight period. These observations agree well with those of Berrill (1950), and Na & Lee (1977), who reported that *C. intestinalis* eggs hatched about 25 hours after fertilisation and the larvae swam for 6-36 hours. (Larvae were observed to hatch by 1700h on the day of spawning when failure of cooling water flow allowed the water temperature in the hatching beaker to rise to 24°C).

Svane & Haverhand (1990; 1993) observed that, in addition to freely spawned eggs, *C. intestinalis* also released ova in strings of mucus which adhered to adults where fertilisation and development proceeded as for free eggs. Newly hatched larvae could either escape into the water column or be retained in the string of mucus where settlement then took place. Strings of eggs were not observed during the present experiments, but any formed would probably have been disrupted by the filtering process.

At water temperatures of 16-20°C, the larvae of *A. aspersa* hatched about early to mid-afternoon on the day after spawning. The larvae were active for seven to eight hours and must therefore have been competent towards the end of the daylight period. If *A. aspersa* larvae respond to light as a behavioural cue, typical pre-settlement behaviour is most likely to be demonstrated by mature larvae exposed to a decreasing light flux.

At water temperatures of 16-20°C, the larvae of *S. clava* hatched about mid-morning on the day after spawning and were active for at least ten hours. So these larvae were also competent towards the end of the daylight period and, if light is a behavioural cue, typical pre-settlement behaviour is most likely to be demonstrated by mature larvae exposed to a decreasing light flux. Given the preference, or requirement, for recruitment in a sheltered habitat, the short life span of these larvae may account for the limited spread of *S. clava*.

The results indicate that light is a potential cue for the larvae of all three species. The spawning and hatching times reported here, together with the observed duration of the larval stage, support the comment made by Svane & Young (1989) that many solitary ascidians attain metamorphic competency at about the same time of day, but accomplish it by different means.

CHAPTER 7 BUOYANCY

7.1 Introduction

An inanimate particle introduced into a column of liquid will exhibit buoyancy. If it is positively buoyant it will float and if it is negatively buoyant it will sink, the direction of its vertical movement being dependent upon its density relative to that of the surrounding medium. The rate of displacement in a liquid of constant temperature, viscosity and density, is determined by the balance between the particle's density and its drag, the latter being a function of cross sectional area. A living particle suspended in sea water will also exhibit buoyancy, but in this case the direction and rate of passive vertical movement may be modified by morphological (e.g. size and shape) and biochemical (e.g. protein/lipid ratio) adaptations. Control of vertical movement by living aquatic organisms results from modulation of the effects of buoyancy by active means. An understanding of the processes which regulate vertical distribution must, therefore, begin with a consideration of buoyancy.

In the context of ascidian settlement, buoyancy can contribute to the final outcome through at least three routes, each of which must be assessed independently. The first question that needs to be addressed is "What is the buoyancy status of the eggs?" The answer to this question will indicate the starting position for newly hatched larvae. A review of the literature indicates some disagreement concerning the buoyancy of *A. aspersa* eggs; Knaben (1952) reported that ripe eggs floated in water of salinity 28‰ or more, whereas Holmes (1968) observed that they sank in water of salinity 33.1‰ even when gentle aeration was applied. Floating eggs would appear to be a dubious strategy as the eggs would incur greater risk of damage by ultra-violet radiation. In the present study the majority of eggs

will be fertilised, and the buoyancy of zygotes may be different to that of ripe eggs. Consequently the buoyancy of fertilised eggs of all three species needs to be assessed.

The next question is "What is the buoyancy status of the larvae?" The answer to this question will allow assessment of the contribution of the active behavioural component to the observed larval position in the water column. The final question is "Does the buoyancy status of the larvae change with age, particularly during the competent period prior to settlement?" The importance of this question will depend upon how pre-settlement larvae respond to the environmental cues.

7.2 Methods

7.2.1 Buoyancy of eggs

Eggs were harvested and maintained in beakers of aerated filtered (10 μm) sea water (salinity 33-34‰). Batches of eggs were decanted into filtered (10 μm) aerated sea water (350 ml). The behaviour chamber and variable head tube (see section 5.5) were filled with filtered (10 μm) sea water, the bottom valve closed and the chamber drained. The suspension of eggs was poured into the behaviour chamber and the top sealed with a rubber bung. The chamber was inverted five times to ensure thorough mixing, fixed in the vertical plane and the bottom valve was opened. The experiment was left to equilibrate for twenty minutes, then all valves were closed. The water in each section of tube was decanted, with rinsing, into labelled sample jars, stained and preserved (section 5.2) for later examination. No attempt was made to select eggs of specific ages or development stages for these

experiments. The Initial distribution of the eggs was determined by draining the segments of the tube into labelled sample pots immediately after tube inversion.

7.2.2 Buoyancy of larvae

7.2.2.1 Direction of buoyancy

Larvae were anaesthetised with benzocaine solution[4] (approximately 0. 1%) and poured gently onto the surface of a column of sea water in a perspex tube (1 m long x 5 cm diameter). The movement of the larvae in the water column was observed.

7.2.2.2 Rate of passive movement

Once the direction of passive movement had been established, the rate of passive movement was determined by timing the travel of individual anaesthetised larvae over a 20 cm pathlength. Larvae which showed active movement (twitching movements etc.) were ignored. The experiment was repeated for ten different larvae.

7.2.2.3 Effect of hydrostatic pressure on the buoyancy of young larvae.

Young larvae (less than two hours old) were decanted into 350 ml of filtered (10 μm) sea water, anaesthetised with benzocaine solution (approximately 0.1%) and the suspension of larvae poured gently into the prepared vertical behaviour chamber. The top of the chamber was sealed with a rubber bung, the pressure set by adjusting the hydrostatic head and the bottom valve opened. The rate of passive movement of the slowest sinking larvae was estimated as approximately fifteen minutes per metre with no applied hydrostatic pressure; an increase in pressure will increase the viscosity of the medium and hence decrease the

[4] A solution of ethyl p-aminobenzoate in ethanol (1 mg ml^{-1}).

sinking rate of the larvae, so the experiment was left to equilibrate for one hour. The valves were then closed, the water in each section of tube was decanted, with rinsing, into labelled sample jars, stained and preserved (section 5.2) for later examination.

7.2.2.4 Effect of hydrostatic pressure on the buoyancy of competent larvae.

Mature larvae (over four hours old) were decanted into 350 ml of filtered (10 μm) sea water, anaesthetised with benzocaine solution (approximately 0.1%) and the suspension of larvae poured gently into the prepared vertical behaviour chamber. The top of the chamber was sealed with a rubber bung, the pressure set by adjusting the hydrostatic head and the bottom valve opened. The experiment was left to equilibrate for one hour and the valves closed. The water in each section of tube was decanted, with rinsing, into labelled sample jars, stained and preserved (section 5.2) for later examination.

7.3 Results

7.3.1 Buoyancy of eggs

The eggs of all three species were found to be negatively buoyant. For each species, the initial distribution of eggs (control) was significantly different ($p<0.005$, *G*-test) from all other distributions after twenty minutes, with and without applied hydrostatic pressure (Tables 20, 21 and 22). For each species, the distribution observed in the absence of applied hydrostatic pressure was significantly different ($p<0.005$, *G*-test) from the distributions with applied hydrostatic pressure.

Table 20 **Distribution (and %) of *S. clava* eggs in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

	Control	Pressure (m head of water)			
		0	1.0	2.0	3.5
Section A (top)	198 (17.3)	63 (2.5)	157 (3.5)	228 (4.1)	118 (2.5)
Section B	223 (19.5)	106 (4.2)	301 (6.7)	336 (6.6)	806 (17.1)
Section C	240 (20.9)	91 (3-6)	503 (11.2)	1227 (22.1)	1065 (22.6)
Section D	259 (22.6)	207 (8.2)	463 (10.3)	1155 (20.8)	1088 (23.1)
Section E (bottom)	226 (19.7)	2058 (81.5)	3068 (68.3)	2576 (46.5)	1635 (34.7)
Number of eggs	1,146	2,525	4,492	5,552	4,712

Table 21 **Distribution (and %) of *C intestinalis* eggs in the vertical behaviour chamber under a variety of hydrostatic pressure conditions**

	Control	Pressure (m head of water)				
		0	1.0	2.0	3.0	3.5
Section A (top)	864 (17.0)	195 (3.4)	106 (1.5)	96 (2.1)	57 (2.6)	49 (2.3)
Section B	1041 (20.5)	155 (2.7)	488 (6.9)	247 (5.4)	152 (6.9)	151 (7.1)
Section C	986 (19.4)	155 (2.7)	601 (8.5)	595 (13.0)	298 (13.5)	247 (11.6)
Section D	1082 (21.3)	325 (5.7)	792 (11.2)	649 (14.2)	214 (9.7)	296 (13.9)
Section E (bottom)	1107 (21.8)	4893 (85.5)	5082 (71.9)	2987 (65.3)	1483 (67.3)	1385 (65.1)
Number of eggs	5,080	5,723	7,069	4,574	2,204	2,128

Table 22 **Distribution (and %) of *A. aspersa* eggs in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

	Control	Pressure (m head of water)			
		0	1.0	2.0	3.5
Section A (top)	151 (19.2)	388 (5.3)	19 (1.9)	34 (1.3)	4 (0.3)
Section B	144 (18.3)	205 (2.8)	36 (3.6)	241 (9.1)	46 (3.1)
Section C	162 (20.6)	293 (4.0)	101 (10.2)	243 (9.2)	186 (12.6)
Section D	150 (19.1)	315 (4.3)	49 (4.9)	219 (8.3)	180 (12.2)
Section E (bottom)	179 (22.8)	6122 (83.6)	788 (79.4)	1906 (72.1)	1060 (71 8)
Number of eggs	786	7,323	993	2,643	1,476

There was a tendency for *S. clava* eggs to become less negatively buoyant with increasing applied hydrostatic pressure (Table 20). The distribution observed with each applied hydrostatic pressure was significantly different ($p<0.005$, *G*-test) from all the distributions observed with all other applied hydrostatic pressures.

Eggs of *C. intestinalis* and *A. aspersa* exhibited a similar, but less pronounced, trend (Tables 21 and 22). The distribution of *C. intestinalis* eggs observed under 2 m applied hydrostatic pressure was significantly different ($p<0.01$, *G*-test) from the distribution observed under 3.5 m applied hydrostatic pressure. All other distributions of *C. intestinalis* eggs were significantly different ($p<0.005$, *G*-test) from each other, irrespective of the applied hydrostatic pressure. All distributions of *A. aspersa* eggs were significantly different ($p<0.005$, *G*-test) from each other.

7.3.2 Direction and rate of passive movement

The larvae of all three species exhibited negative buoyancy. They sank with the head positioned downward. The passive rate of sinking for each species is presented in Table 23. A mixed age range of larvae was used in these experiments.

Table 23 Passive sinking rates of ascidian larvae (± sd)

	Sinking rate (cm s^{-1})		
Observation No.	***C intestinalis***	***A. aspersa***	***S. clava***
1	0.128	0.178	0.167
2	0.204	0.193	0.177
3	0.157	0.161	0.239
4	0.254	0.204	10.172
5	0.153	0.188	0.257
6	0.144	0.208	0.345
7	0.137	0.172	0.253
8	0.165	0.214	0.262
9	0.183	0.177	0.239
10	0.172	0.184	0.177
Mean	0.170 ± 0.037	0.188 ± 0.017	0.229 ± 0.056

7.3.3 Buoyancy of larvae

After one hour, the distributions of anaesthetised larvae in the vertical behaviour chamber without applied hydrostatic pressure was significantly different ($p<0.005$, *G*-test) from the initial distributions for all three species (Tables 24-29). These results support the initial observation that the larvae are negatively buoyant.

The effect of hydrostatic pressure on larval buoyancy was different for each species. Young *C. intestinalis* larvae showed no clear trend in variation of buoyancy with hydrostatic

pressure (Table 24), although all the larval distributions were significantly different ($p<0.05$, *G*-test) for each other. Mature *C intestinalis* larvae also showed no clear trend in variation of buoyancy with hydrostatic pressure (Table 25), although all of these distributions were significantly different from each other ($p<0.05$, *G*-test).

With the exception of the distributions resulting from exposure to 1.5 m hydrostatic pressure, the proportion of young anaesthetised larvae found in the bottom section of the behaviour chamber was lower than that of mature larvae.

Young *A. aspersa* larvae became less negatively buoyant as hydrostatic pressure increased from 0 m to 2 m head of water; the tendency to negative buoyancy increased again at hydrostatic pressures greater than 2 m head of water (Table 26). All distributions of young larvae were significantly different ($p<0.05$, *G*-test) from each other. Mature *A. aspersa* larvae showed a similar variation of buoyancy with hydrostatic pressure (Table 27), with all larval distributions significantly different ($p<0.05$, *G*-test) from each other. The distributions of young and old larvae after exposure to a given hydrostatic pressure were significantly different ($p<0.05$, *G*-test), but the proportion of young anaesthetised larvae found in the bottom section of the behaviour chamber was not always lower than that of mature larvae.

Both young and mature larvae of *S. clava* became more negatively buoyant as hydrostatic pressure increased from 0 m to 3.5 m of water (Tables 28 & 29). All distributions were significantly different ($p<0.05$, *G*-test) from each other. With the exception of the distributions resulting from exposure to 3.0 m hydrostatic pressure, the proportion of young anaesthetised larvae found in the bottom section of the behaviour chamber was lower than that of mature larvae.

Table 24 **The distribution (and %) of anaesthetised young *C. intestinalis* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

		Hydrostatic pressure (m head of water)							
	Control	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**
Section A (top)	177 (20.8)	31 (3.0)	57 (10.9)	22 (3.7)	57 (3.7)	152 (3.4)	49 (9.1)	317 (5.4)	51 (5.0)
Section B	150 (17.6)	107 (10.4)	45 (8.6)	35 (5.9)	99 (6.5)	356 (7.9)	68 (12.6)	429 (7.4)	57 (5.6)
Section C	148 (17.4)	99 (9.6)	34 (6.5)	44 (7.4)	102 (6.7)	469 (10.4)	27 (5.0)	589 (10.1)	105 (10.3)
Section D	196 (22.9)	162 (15.8)	74 (14.1)	87 (14.7)	182 (11.9)	611 (13.5)	73 (13.5)	781 (13.4)	118 (11.6)
Section E	182 (21.3)	627 (61.1)	314 (59.9)	405 (68.3)	1092 (71.3)	2923 (64.8)	322 (59.7)	3709 (63.7)	687 (67.5)
Number of larvae	853	1026	524	593	1532	4511	539	5825	1018

Table 25 **The distribution (and %) of anaesthetised mature *C. intestinalis* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

		Hydrostatic pressure (m head of water)							
	Control	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**
Section A (top)	177 (20.8)	4 (1.0)	35 (3.7)	187 (3.1)	36 (1.4)	35 (1.3)	41 (3.1)	25 (2.4)	13 (0.4)
Section B	150 (17.6)	51 (12.4)	42 (4.5)	394 (6.4)	101 (4.0)	96 (3.6)	86 (6.5)	49 (4.7)	76 (2.6)
Section C	148 (17.4)	22 (5.4)	62 (6.6)	460 (7.5)	270 (10.8)	262 (9.9)	131 (9.8)	85 (8.1)	191 (6.6)
Section D	196 (22.9)	57 (13.9)	191 (20.3)	762 (12.5)	328 (13.2)	438 (16.5)	217 (16.3)	153 (14.6)	602 (20.7)
Section E	182 (21.3)	276 (67.3)	611 (64.9)	4308 (70.5)	1759 (70.5)	1818 (68.6)	856 (64.3)	734 (70.2)	2030 (69.7)
Number of larvae	853	410	941	6111	2494	2649	1331	1046	2912

Table 26 **The distribution (and %) of anaesthetised young *A. aspersa* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

		Hydrostatic pressure (m head of water)							
	Control	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**
Section A (top)	153 (18.1)	22 (0.9)	89 (2.7)	42 (0.9)	71 (3.6)	383 (9.8)	41 (9.7)	49 (1.2)	28 (2.6)
Section B	194 (22.9)	161 (6.5)	108 (3.3)	137 (3.0)	288 (14.5)	375 (9.6)	39 (9.2)	127 (3.1)	27 (2.5)
Section C	198 (23.4)	119 (4.8)	135 (4.1)	170 (3.7)	272 (13.7)	496 (12.7)	14 (3.3)	217 (5.3)	46 (4.3)
Section D	147 (17.4)	185 (7.4)	299 (9.1)	375 (8.3)	191 (9.6)	793 (20.3)	44 (10.4)	356 (8.7)	87 (8.2)
Section E	154 (18.2)	2005 (80.5)	2653 (80.8)	3812 (84.0)	1160 (58.5)	1858 (47.6)	286 (67.5)	3347 (81.7)	876 (82.3)
Number of larvae	846	2492	3284	4536	1982	3905	424	4096	1064

Table 27 **The distribution (and %) of anaesthetised mature *A. aspersa* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.**

		Hydrostatic pressure (m head of water)							
	Control	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**
Section A (top)	153 (18.1)	51 (5.9)	24 (2.6)	25 (4.1)	66 (4.6)	123 (10.0)	171 (8.9)	60 (2.1)	8 (1.4)
Section B	194 (22.9)	42 (4.8)	37 (4.1)	12 (2.0)	128 (9.0)	149 (12.1)	98 (5.1)	76 (2.7)	21 (3.6)
Section C	198 (23.4)	22 (2.5)	35 (3.9)	30 (4.9)	167 (11.7)	114 (9.2)	153 (8.0)	140 (5.0)	26 (4.5)
Section D	147 (17.4)	34 (3.9)	73 (8.0)	62 (10.2)	164 (11.5)	234 (18.9)	192 (10.0)	276 (9.8)	54 (9.3)
Section E	154 (18.2)	717 (82.8)	740 (81.4)	478 (78.7)	904 (63.3)	615 (49.8)	1308 (68.1)	2267 (80.5)	472 (81.2)
Number of larvae	846	866	909	607	1429	1235	1922	2828	581

Table 28 The distribution (and %) of anaesthetised young *S. clava* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.

		Hydrostatic pressure (m head of water)							
	Control	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Section A (top)	489 (16.1)	35 (3.7)	161 (3.2)	145 (3.6)	349 (3.5)	210 (1.7)	74 (1.7)	398 (1.7)	13 (1.5)
Section B	708 (23.4)	39 (4.1)	216 (4.3)	153 (3.8)	307 (3.1)	388 (3.2)	96 (2.2)	543 (2.4)	19 (2.2)
Section C	679 (22.4)	77 (8.1)	416 (8.3)	298 (7.4)	360 (3.6)	399 (3.3)	227 (5.2)	9.8 (3.9)	25 (2.9)
Section D	566 (18.7)	177 (18.5)	843 (16.8)	528 (13.1)	1114 (11.2)	1172 (9.7)	336 (7.7)	1550 (6.7)	76 (8.8)
Section E	588 (19.4)	628 (65.7)	3381 (67.4)	2904 (72.1)	7789 (78.5)	9852 (82.0)	3613 (83.1)	19628 (85.2)	733 (84.6)
Number of larvae	3030	956	5017	4028	9919	12021	1346	23027	866

Table 29 The distribution (and %) of anaesthetised mature *S. clava* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions.

		Hydrostatic pressure (m head of water)							
	Control	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Section A (top)	489 (16.1)	73 (3.5)	118 (3.9)	175 (3.3)	128 (2.1)	36 (1.3)	80 (1.3)	44 (1.2)	89 (0.9)
Section B	708 (23.4)	101 (4.8)	154 (5.1)	186 (3.5)	171 (2.8)	61 (2.2)	116 (1.9)	76 (2.1)	462 (4.6)
Section C	679 (22.4)	159 (7.5)	241 (8.0)	335 (6.3)	282 (4.6)	112 (4.0)	171 (2.8)	127 (3.5)	468 (4.7)
Section D	566 (18.7)	361 (17.1)	419 (13.9)	574 (10.8)	608 (9.9)	237 (8.5)	570 (9.3)	326 (9.0)	460 (4.6)
Section E	588 (19.4)	1415 (67.1)	2090 (69.2)	4046 (76.1)	4962 (80.7)	2346 (84.0)	5187 (84.7)	3053 (84.2)	8564 (85.3)
Number of larvae	3030	2109	3022	5316	6151	2792	6124	3626	10043

7.4 Discussion

The three ascidian species studied in this project are oviparous; the eggs are spawned into the water, where they are fertilised, undergo maturation and complete development to the swimming larval stage. For these species the major part of the dispersal phase is passed in the egg and embryonic stages, the duration of which will be temperature dependent.

Dispersion is passive during these stages, but it can be aided by one or more properties of the egg. The sinking rate of a sphere at low Reynolds number, such as an ascidian egg, is determined by the balance between its density and its drag, the latter being a function of cross-sectional area. If the drag can be increased without a proportional increase in density, the egg will sink more slowly (Vogel, 1981; Chia *el al.*, 1984). For ascidian eggs this is accomplished in four ways. First, eggs may undergo osmotic expansion of the perivitelline space shortly after contacting sea water (Berrill, 1928; 1975). Second, drag can be increased by projections on the outside of the egg which effectively increase the cross-sectional area of the egg, e.g. *C. intestinalis* (Berrill, 1947). Third, additional diameter is conferred by the follicle cells, which are heavily vacuolated (hence light) cells that cover the outside of the chorion. Finally, the follicle cells of a few species (e.g. *A. aspersa, Corella inflata)* are lighter than sea water and cause the eggs to float; in the case of *C. inflata* the buoyancy is due to ammonium ions in the follicle cells (Lambert & Lambert, 1979).

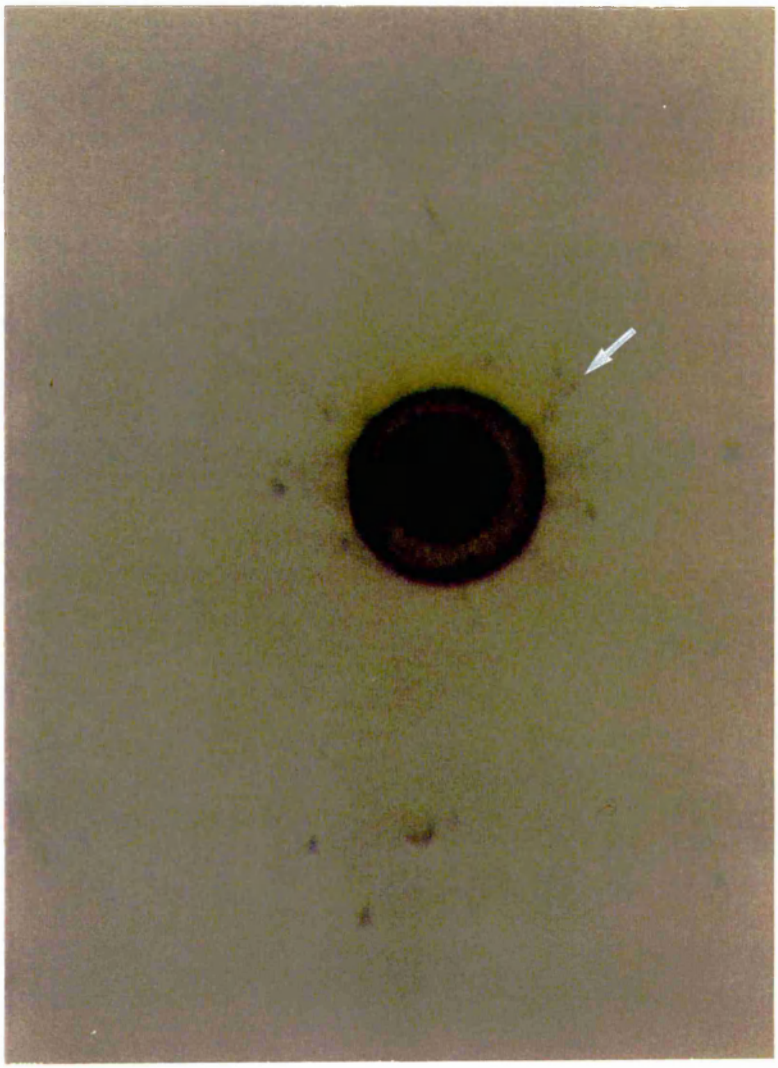
Despite the presence of adaptations to buoy-up the eggs, it should be advantageous for freshly fertilised eggs to sink some way down in the water column away from direct sunlight. During the first phase of myoplasmic segregation, axial and muscle determinants move to the vegetal pole region of the uncleaved ascidian zygote. At this stage the axial

determinants can be inactivated by ultra-violet (UV) radiation (Jeffery, 1990); irradiation of *S. clava* eggs between the first and second phases of ooplasmic segregation suggests that the UV-sensitive component of the axial determinant system may be nucleic acid (Jeffery, 1994). Thus it will be favourable for *S clava* survival if zygotes in the early stages of development sink away from the influence of intense UV radiation so that normal development can occur and genetic material can be transmitted with minimum disruption. In the absence of any indication to the contrary in the literature, it is reasonable to assume that the zygotes of *A. aspersa* and *C. intestinalis* are similarly affected by UV radiation. The sinking of eggs would be a simple strategy to avoid genetic damage by UV radiation.

The eggs of all three species sank in these experiments. No attempt was made to control the age, and therefore the development stage, of the eggs used in the experiments. It was considered that the size and density of the eggs would vary little after the first hour, when osmotic expansion should have been complete. The conditions in these experiments were artificial as the water in the vertical chamber was still whereas in the natural situation water movement due to wind and waves would tend to buoy the eggs up in the water column. Indeed, Berrill (1947) reported that *C. intestinalis* eggs sank in still water but buoyant outer follicle cells kept them in suspension "with the slightest agitation". In this study, projections similar to the "viliform" follicle cells reported by Na & Lee (1977) were observed on *C. intestinalis* eggs (Plate 42) which would act to prolong buoyancy in non-static water.

Lack of water movement may account for the sinking of *A. aspersa* eggs; ripe eggs have previously been reported to float in sea water of salinity greater than 30‰ (Berrill, 1928) and 28‰ (Knaben, 1952), although Holmes (1968) observed *A. aspersa* eggs to sink even with aeration and consequent turbulence. It is possible that sinking of *A. aspersa* eggs could

PLATE 42 ***Ciona intestinalis*** **egg showing follicle cell projections**



be due to damage or removal of the follicle cells; Lambert & Lambert (1979) and Young (1988) easily removed the follicle cells by shaking the eggs vigorously in a centrifuge tube. Although the eggs in the present study were treated with all possible care, filtration with particles of detritus could have damaged some follicle cells.

The larvae of all three species are negatively buoyant but the effect of hydrostatic pressure on buoyancy is different for each species. The differences are most apparent when the proportions of larvae found in the bottom section of the vertical behaviour chamber are compared. Increases in hydrostatic pressure did not appear to influence the proportion of *C. intestinalis* larvae exhibiting negative buoyancy (Figure 13). However, the proportion of *A aspersa* larvae exhibiting negative buoyancy decreased as hydrostatic pressure increased, reaching a minimum at 2 m head of water, then increased again as pressure increased further (Figure 14). Thus the effect of negative buoyancy on the larval population is minimal at 2 m depth, and minimum energy will be expended to maintain station at this depth in the water column; so it is energetically favourable for *A. aspersa* to maintain position at this depth.

The proportion of *S. clava* larvae exhibiting negative buoyancy increased as hydrostatic pressure increased (Figure 15) so the influence of negative buoyancy on the larvae is at a minimum at the surface and they have to expend minimum energy to maintain station in the water column if they float near the surface. Thus it is energetically favourable for *S. clava* larvae to remain near the surface.

It is apparent that the effects of negative buoyancy must be overcome if larvae are to maintain vertical position or move upward in the water column. There is no evidence that ascidian larvae can regulate buoyancy directly by morphological or physiological

mechanisms. There are no gas-filled bladders and ability to alter body shape is limited. The papillae of *C. intestinalis* and *A. aspersa* may increase drag and reduce sinking rate, but the effect is probably negligible; *S. clava* larvae have no appendages available to increase drag. The effect of pressure on the buoyancy of *S. clava* larvae suggests the presence of a buoyancy mechanism based on gas inclusion, i.e. the gas is compressed as the pressure increases and the larva becomes less buoyant. On the limited data available no satisfactory explanation can be offered for the effect of pressure on the buoyancy of *A. aspersa* larvae.

In the majority of the experiments, young larvae were more buoyant than mature larvae of the same species, despite the rather arbitrary differentiation of the age groups. The decline in buoyancy may be due to depletion of lipid reserves, and consequent increase in specific gravity, as the lecithotrophic larvae expend energy swimming. If this is the case, negative buoyancy would increase with larval age and could be a contributory factor in the observed tendency of larvae to sink after an initial swim-up period.

The majority of the larval tested were negatively buoyant, but small proportions did not sink to the bottom section of the vertical chamber. Failure to sink was not due to insufficient experimental time, but may have been due to incomplete anaesthetisation or passive physical factors such as the attachment of micro-bubbles (from aeration), convection currents in the water column or vibration of the chamber. The effects of negative buoyancy can be actively modified only by locomotor responses mediated by the behaviour repertoire of the species. Downward movement may be effected by cessation of locomotor activity or by locomotion complementing negative buoyancy; upward movement, however, can be achieved only by active locomotion, oriented such that it opposes the effect of negative buoyancy. The following chapters examine the larval behaviour that modifies buoyancy.

Figure 13 Buoyancy of mature C. intestinalis larvae

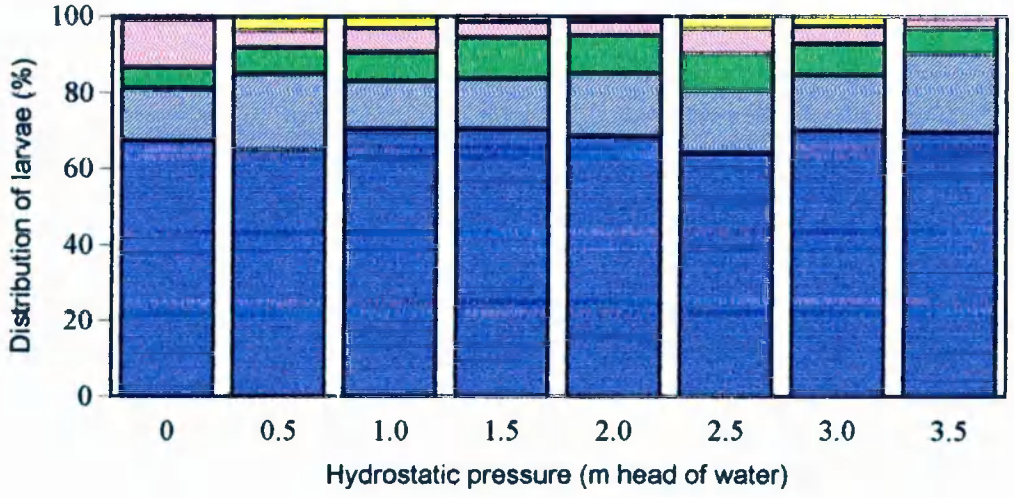


Figure 14 Buoyancy of mature A. aspersa larvae

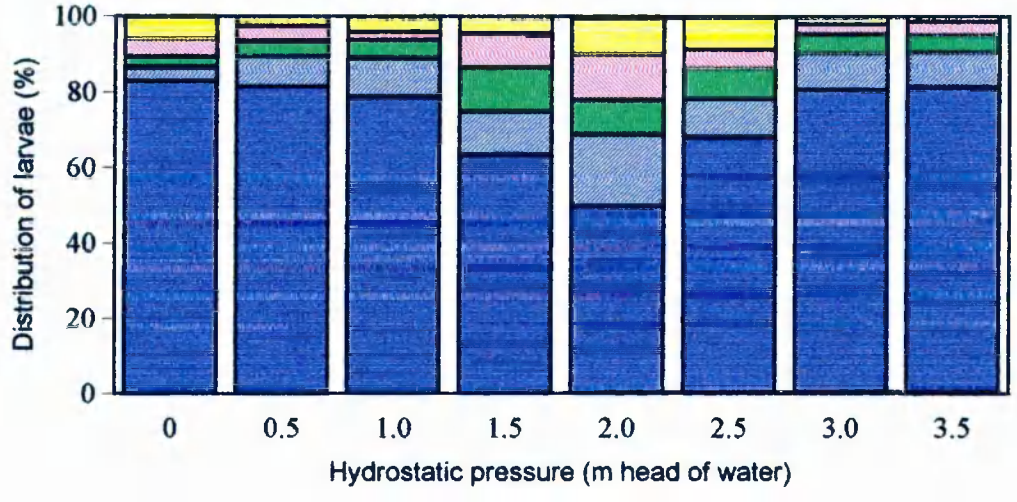
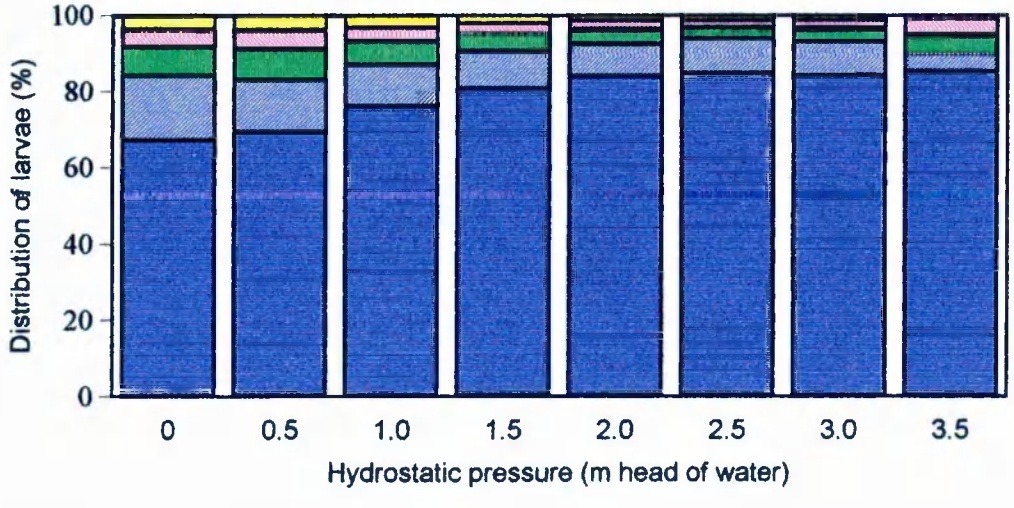


Figure 15 Buoyancy of mature S. clava larvae



See Figure 10 (page 88) for convention used in Figures 13-15.

CHAPTER 8 LARVAL RESPONSE TO GRAVITY

8.1 Introduction

Gravity is a conservative orientation stimulus which acts in the vertical plane. It is ubiquitous, unidirectional and, in the framework of this study, constant in intensity. Movement along lines of gravitational force is termed geotaxis (Fraenkel & Gunn, 1961). By convention, positive geotaxis is directed towards the centre of the earth and negative geotaxis away from it. Many ascidian larvae possess either statocysts or statocytes that contain granules of black melanin pigment (Whittaker, 1966) and are thought to function in the detection of gravity (Berrill, 1975; Eakin & Kuda, 1971), but apparent geotaxis has been observed both in larvae that are known to have gravity receptors and those that do not (Crisp & Ghobashy, 1971). Bryozoan larvae lack statocysts, yet some exhibit an apparent negative geotaxis prior to settlement (Pires & Woollacott, 1983). To date there is no direct evidence that larval movement is accomplished through an active, direct response to gravity.

Experimental methods used to quantify orientation responses usually involve measuring shifts in distribution of a sample of larvae which have been stimulated by the environmental cue in question. Orientation is thus inferred and it is essential to manipulate the experimental system in a way that can produce unequivocal results. This can be particularly difficult when testing the response to gravity, since changes in distribution in the vertical plane can be confounded by buoyancy effects. For example, if net shift in distribution is downward, the result could be attributed solely to negative buoyancy, to a combination of negative buoyancy and positive geotaxis, or to the dominance of passive sinking over locomotor response in negatively geotactic larvae.

Because of these complexities, results from experiments which measure only shifts in distribution in vertically orientated observation tanks (e.g. Holmes, 1968) should be interpreted with caution; such experiments should be supplemented with additional data. Ott & Foward (1976) and Latz & Foward (1977) compared passive sinking rates with downward movement of unanaesthetized crustacean larvae. If the rate of downward movement exceeded that of passive sinking, positive geotaxis was inferred. Sulkin *et al.* (1980) supplemented a vertically-orientated chamber with an identical chamber positioned horizontally. Crustacean larval movement in darkness along the axis of the horizontal chamber provided a measure of random (non-orientated) activity, while movement in darkness along the axis of the vertical chamber reflected both random and oriented responses. If upward movement along the axis of the vertical chamber exceeded movement in the horizontal chamber, they inferred negative geotaxis; if movement in the vertical chamber was less than that which could be attributed to random activity alone (horizontal chamber), positive geotaxis was inferred. However, it should be noted that there could be a barokinesis component in such results, depending upon the hydrostatic pressure in the vertical behaviour chamber, which would not be present in the horizontal chamber.

Few direct studies have been carried out on the effect of gravity. Pires & Woollacott (1983) tested for a direct effect of gravity on the settlement of *Bugula* larvae by inducing the larvae to settle on wooden substrata inside buckets swung in a low speed centrifuge. As such equipment was not readily available to me, I attempted to balance out the effect of gravity by rotating containers in the vertical plane. Jars containing fixed, roughened plastic strips and ascidian larvae in filtered (10 μm) sea water were attached to a vertical rotating disc such that they were continually inverting. However, few larvae settled, probably because it was difficult to completely remove air from the jars so that small air bubbles were

intermittently moving the length of the jars. Such experiments would, in any case, only give an indication of settlement behaviour when the effect of gravity was balanced out. There would be no indication if larval response prior to settlement was different, so this would still have to be inferred.

As the more direct method failed, I resorted to the traditional approach of inference to determine the response of the larvae to gravity. However, I employed both of the techniques outlined earlier in an attempt to obtain a fuller understanding of the effect of gravity on pre-settlement larvae. Larval distribution in the vertical tube in the absence of light was compared with the buoyancy of anaesthetised larvae and with the distribution of larvae in darkness in the horizontal behaviour chamber. This approach assumes that the effects of barokinesis, temperature gradient and gradient of partial pressure of dissolved gasses will be minimal in the narrow 1 m column of water. The effect of hydrostatic pressure on the response of larvae to gravity will be examined in a separate chapter.

Many previous studies have noted that larvae swim up immediately after hatching (see for example Cloney, 1987). Therefore I have attempted a comparison of the response to gravity of young, recently hatched larvae (< 2h old) with mature larvae (> 4h old).

8.2 Methods

8.2.1 Vertical behaviour chamber

The vertical behaviour chamber was prepared, and larvae introduced, as described in section 5.5. A rubber bung was inserted into the top of the chamber to exclude light, the chamber

was inverted five times, then fixed in the vertical plane. The hydrostatic head was adjusted to 0 m by raising the open end of the polythene tube until the water level in it was level with the top of the vertical chamber; the connecting valve was then opened. The chamber was left at constant temperature for one hour, the valves were closed and the segments of water were decanted, with rinsing, into labelled sample pots. The samples were stained and preserved for later examination (section 5.2). The number of larvae present in each sample was determined by direct counting and the results compared with the buoyancy of larvae at 0 m hydrostatic pressure previously determined (Chapter 7).

Control experiments were carried out to determine the initial distribution of the larvae in the behaviour chamber. Larvae were introduced into the chamber as above, but the segments were isolated and drained immediately after mixing (section 5.5).

8.2.2 Horizontal behaviour chamber

A cover was placed over the perspex window of the horizontal behaviour tube (section 5.6) to exclude light. The chamber was prepared and larvae introduced through the middle entry port as described in section 5.6. The tube was left for one hour at constant temperature, then rotated. The segments of water were drained, with rinsing (10 μm filtered sea water) into labelled sample pots and preserved for later examination (section 5.2). The number of larvae present in each sample was determined by direct counting.

Control experiments were carried out with live larvae of each species and dead, preserved and stained *C. intestinalis* larvae (section 5.6).

8.3 Results

8.3.1 *Ciona intestinalis* larvae

8.3.1.1 Vertical behaviour chamber

In the absence of light and applied hydrostatic pressure, 15-30% of the population of active young *C. intestinalis* larvae sank to the bottom section of the vertical chamber whilst 33-37% rose to the top section (Table 30). When anaesthetised (section 7.3.3), over 60% of the population of young larvae sank to the bottom section of the chamber whilst only 3% rose to the top section (Table 30). A similar proportion of the population of active mature larvae sank to the bottom section of the vertical chamber (Table 31), but the proportion that rose to the top section was smaller (22-26%). When anaesthetised 67% of the population of mature larvae sank to the bottom section of the vertical chamber (Table 31).

Table 30 Distribution (and %) of young *C. intestinalis* larvae in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	**Expt. I**	**Expt. 2**	**Expt. 3**	**Buoyancy (Chapt. 7)**
Section A (top)	177 (20.8)	395 (37.3)	1174 (33.4)	1454 (33.2)	31 (3.0)
Section B	150 (17.6)	140 (13.2)	625 (17.8)	662 (15.1)	107 (10.4)
Section C	148 (17.4)	137 (12.9)	667 (19.0)	452 (10.3)	99 (9.6)
Section D	196 (23.0)	130 (12.3)	522 (14.9)	536 (12.2)	162 (15.8)
Section E (bottom)	182 (21.3)	258 (24.3)	522 (14.9)	1276 (29.1)	627 (61.1)
Total number of larvae	853	1060	3510	4380	1026

Table 31 Distribution (and %) of mature *C. intestinalis* in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	Expt. I	Expt. 2	Expt. 3	Buoyancy (Chapt. 7)
Section A (top)	177 (20.8)	221 (26.1)	405 (22.7)	201 (23.8)	4 (1.0)
Section B	150 (17.6)	242 (28.6)	281 (15.7)	208 (24.6)	51 (12.4)
Section C	148 (17.4)	127 (15.0)	330 (18.5)	117 (13.8)	22 (5.4)
Section D	196 (23.0)	118 (13.9)	235 (13.2)	111 (13.1)	57 (13.9)
Section E (bottom)	182 (21.3)	139 (16.4)	536 (30.0)	208 (24.6)	276 (67.3)
Total number of larvae	853	847	1787	845	410

All distributions of active larvae are significantly different ($p<0.005$, *G*-test) from the initial larval distribution, as indicated by the control experiment, and from the distributions of anaesthetised larvae at 0 m applied hydrostatic pressure. The distributions of young and mature larvae are also significantly different ($p<0.005$, *G*-test) from each other. The results indicate that larvae swim up in the absence of light, opposing and exceeding the effect of negative buoyancy. The response is greater for young larvae than for mature larvae.

8.3.1.2 Horizontal behaviour chamber

In the absence of light and applied hydrostatic pressure, the distribution of both young and mature *C. intestinalis* larvae after one hour was similar to the dead control and the immediately removed live control (Tables 32 and 33). However, statistical analysis indicated that the distributions of live and dead controls were significantly different ($p<0.005$, *G*-test), and all experimental distributions were significantly different ($p<0.005$, *G*-test) from both

control distributions. Thus, although distribution changes were small, live larvae did disperse horizontally.

Table 32 Distribution (and %)) of young *C. intestinalis* in the horizontal behaviour chamber in the absence of light and hydrostatic pressure.

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	343 (4.7)	0 (0)	733
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	34 (6.4)	208 (39.0)	225 (42.2)	55 (10.3)	11 (2.1)	533
Expt. 2	44 (6.1)	263 (36.6)	296 (41.2)	87 (12.1)	28 (3.9)	718
Expt. 3	203 (6.2)	1242 (37.5)	1506 (45.5)	211 (6.4)	146 (4.4)	3308

Table 33 Distribution (and %)) of mature *C. intestinalis* in the horizontal behaviour chamber in the absence of light and hydrostatic pressure.

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	343 (4.7)	0 (0)	733
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	53 (4.0)	469 (35.4)	625 (47.2)	133 (10.0)	45 (3.4)	1325
Expt. 2	153 (7.4)	608 (29.5)	983 (47.8)	204 (9.9)	110 (5.3)	2058
Expt. 3	232 (4.2)	1874 (33.6)	2807 (50.3)	481 (8.6)	185 (3.3)	5579

All the distributions of young larvae were significantly different ($p<0.05$, *G*-test) from each other. All the distributions of mature larvae were significantly different ($p<0.005$, *G*-test) from each other. All the distributions of young larvae were significantly different ($p<0.05$, *G*-test) from the distributions of mature larvae.

8.3.2 *Ascidiella aspersa*

8.3.2.1 Vertical behaviour chamber

In the absence of light and applied hydrostatic pressure, 20-25% of the population of active young *A. aspersa* larvae sank to the bottom section of the vertical chamber whilst 36-44% rose to the top section (Table 34), but over 80% of the population of anaesthetised larvae sank to the bottom section of the vertical chamber (section 7.3.3). A larger proportion (48-55%) of the population of active mature *A. aspersa* larvae sank to the bottom section of the vertical chamber (Table 35), with a commensurately smaller proportion rising to the top section (9-23%). The comparable response for anaesthetised mature larvae was 83% sinking to the bottom section of the vertical chamber. All distributions of active larvae are significantly different ($p<0.005$, *G*-test) from the initial larval distribution, as indicated by the control experiment, and the distributions of anaesthetised larval at 0 m applied hydrostatic pressure. The distributions of young and mature larvae are significantly different ($p<0\ 005$, *G*-test) from each other.

Table 34 Distribution (and %) of young *A. aspersa* in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	**Expt. I**	**Expt. 2**	**Expt. 3**	**Buoyancy (Chapt. 7)**
Section A (top)	153 (18.1)	438 (43.6)	188 (36.6)	183 (42.1)	22 (0.9)
Section B	194 (22.9)	109 (10.8)	106 (20.6)	76 (15.5)	161 (6.5)
Section C	198 (23.4)	125 (12.4)	48 (9.3)	47 (10.8)	119 (4.8)
Section D	147 (17.4)	129 (12.8)	43 (8.4)	41 (9.4)	185 (7.4)
Section E (bottom)	154 (18.2)	204 (20.3)	129 (25.1)	88 (20.2)	2005 (80.5)
Total number of larvae	846	1005	514	435	2492

Table 35 Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	Expt. I	Expt. 2	Expt. 3	Buoyancy (Chapt. 7)
Section A (top)	153 (18.1)	288 (22.5)	32 (17.9)	36 (9.4)	51 (5.9)
Section B	194 (22.9)	148 (11.6)	23 (12.8)	37 (9.7)	42 (4.8)
Section C	198 (23.4)	119 (9.3)	16 (8.9)	39 (10.2)	22 (2.5)
Section D	147 (17.4)	102 (8.0)	16 (8.9)	59 (15.4)	34 (3.9)
Section E (bottom)	154 (18.2)	621 (48.6)	92 (51.5)	212 (55.4)	717 (82.8)
Total number of larvae	846	1278	179	383	866

The results indicate that in the absence of light larvae swim up, opposing and exceeding negative buoyancy. The response is greater for young larvae than for mature larvae.

8.3.2.2 Horizontal behaviour chamber

In the absence of light and applied hydrostatic pressure, the distribution of both young and mature *A. aspersa* larvae after one hour was similar to the dead control and the immediately removed live control (Tables 36 and 37); but all experimental distributions were significantly different ($p<0.005$, *G*-test) from both control distributions. Thus, although distribution changes were small, live larvae did disperse horizontally. The control distributions were significantly different ($p<0.005$, *G*-test) from each other.

All distributions of young larvae were significantly different ($p<0.005$, *G*-test) from each other, and all distributions of mature larvae were significantly different ($p<0.005$, *G*-test) from each other. All distributions of young larvae were significantly different ($p<0.05$, *G*-test) from the distributions of mature larvae.

Table 36 Distribution (and %)) of young *A. aspersa* in the horizontal behaviour chamber in the absence of light and hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	82 (6.0)	282 (20.6)	651 (47.5)	250 (18.2)	105 (7.7)	1370
Expt. 2	27 (5.2)	147 (28.4)	256 (49.5)	69 (13.3)	18 (3.5)	517
Expt. 3	16 (2.6)	182 (30.2)	262 (43.6)	93 (15.5)	49 (8.1)	602

Table 37 Distribution (and %)) of mature *A. aspersa* in the horizontal behaviour chamber in the absence of light and hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	23 (2.4)	279 (29.6)	480 (50.8)	119 (12.6)	43 (4.6)	944
Expt. 2	71 (3.9)	594 (32.4)	796 (43.4)	256 (14.0)	115 (6.3)	1832
Expt. 3	72 (4.6)	481 (30.7)	783 (50.0)	171 (10.9)	59 (3.8)	1556

8.3.3 *Styela clava*

8.3.3.1 Vertical behaviour chamber

In the absence of light and applied hydrostatic pressure, less than 7% of the population of active young *S. clava* larvae sank to the bottom section of the vertical chamber whilst over 78% rose to the top section (Table 38). However, over 67% of the population of

anaesthetised larvae sank to the bottom section of the vertical chamber (section 7.3.3). A larger proportion (up to 14%) of the population of active mature *S. clava* larvae sank to the bottom section of the vertical chamber (Table 38), with a commensurately smaller proportion rising to the top section (50-65%). Anaesthetised mature larvae responded similarly to anaesthetised young larvae with 67% sinking to the bottom section of the vertical chamber.

Table 38 Distribution (and %) of young *S. clava* in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	Expt. I	Expt. 2	Expt. 3	Buoyancy (Chapt. 7)
Section A (top)	489 (16.1)	900 (79.1)	7049 (79.5)	680 (78.7)	35 (3.7)
Section B	708 (23.4)	93 (8.2)	760 (8.6)	88 (10.2)	39 (4.1)
Section C	679 (22.4)	48 (4.2)	345 (3.9)	40 (4.6)	77 (8.1)
Section D	566 (18.7)	22 (1.9)	196 (2.2)	19 (2.2)	177 (18.5)
Section E (bottom)	588 (19.4)	75 (6.6)	522 (5.9)	37 (4.3)	628 (65.7)
Total number of larvae	3030	1138	8872	864	956

Table 39 Distribution (and %) of mature *S. clava* in the vertical behaviour chamber at 0 m hydrostatic pressure in the absence of light.

	Control	Expt. I	Expt. 2	Expt. 3	Buoyancy (Chapt. 7)
Section A (top)	489 (16.1)	154 (50.3)	3324 (64.9)	343 (51.8)	73 (3.5)
Section B	708 (23.4)	51 (16.7)	1101 (21.5)	102 (15.4)	101 (4.8)
Section C	679 (22.4)	30 (9.8)	313 (6.1)	71 (10.7)	159 (7.5)
Section D	566 (18.7)	30 (9.8)	180 (3.5)	53 (8.0)	361 (17.1)
Section E (bottom)	588 (19.4)	41 (13.4)	207 (4.0)	93 (14.0)	1415 (67.1)
Total number of larvae	3030	306	5125	662	2109

All distributions of active larvae are significantly different ($p<0.005$, *G*-test) from the initial larval distribution, as indicated by the control experiment, and from the distributions of anaesthetised larval at 0 m applied hydrostatic pressure. Distributions of young and mature larvae are significantly different($p<0.005$, *G*-test) from each other. These results indicate that larvae swim up in the absence of light, opposing and exceeding the effect of negative buoyancy, and this response is greater for young larvae than for mature larvae.

8.3.3.2 Horizontal behaviour chamber

In the absence of light and applied hydrostatic pressure, the distributions of both young and mature *S clava* larvae were similar to the live and dead controls (Tables 40 and 41), but all experimental distributions were significantly different ($p<0.005$, *G*-test) from the control distributions; so although distribution changes were small, larvae did disperse horizontally. Larval distributions in the controls were significantly different ($p<0.005$, *G*-test).

Table 40 Distribution (and %)) of young *S. clava* in the horizontal behaviour chamber in the absence of light and hydrostatic pressure.

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	2 (0.4)	248 (38.2)	357 (54.9)	42 (6.5)	1 (0.1)	650
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	52 (6.1)	290 (34.2)	354 (41.7)	106 (12.5)	46 (5.4)	848
Expt. 2	28 (4.9)	188 (33.2)	256 (45.2)	74 (13.1)	20 (3.5)	566
Expt. 3	38 (3.4)	413 (36.7)	531 (47.2)	107 (9.5)	35 (3.1)	1124

The distributions of young larvae were significantly different ($p<0.005$, *G*-test) from each other, as were all the distributions of mature larvae. The distributions of young larvae were significantly different ($p<0.05$, *G*-test) from the distributions of mature larvae.

Table 41 Distribution (and %)) of mature *S. clava* larvae in the horizontal behaviour chamber in the absence of light and hydrostatic pressure.

	Section A (light)	Section B	Section C	Section D	Section E (dark)	Number of larvae
Mean live control	2 (0.4)	248 (38.2)	357 (54.9)	42 (6.5)	1 (0.1)	650
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	70 (4.3)	426 (26.3)	862 (53.2)	209 (12.9)	52 (3.2)	1619
Expt. 2	20 (4.9)	135 (32.8)	178 (43.2)	62 (15.0)	17 (4.1)	412
Expt. 3	25 (4.2)	188 (31.5)	273 (45.8)	80 (13.4)	30 (5.0)	596

8.4 Discussion

A substantial proportion of active larvae of all three ascidian species rose up in the vertical behaviour chamber in the absence of light; this vertical movement opposes and exceeds the effect of negative buoyancy and must therefore represent an active response. Only a small proportion of active larvae of the three ascidian species moved away from the area of introduction in the dark horizontal behaviour chamber. Since light and gravity are the only vector cues available to the larvae and neither can be operating as directional cues in the horizontal chamber, the small horizontal dispersion must represent the undirected movement of the larvae. The change in larval distribution brought about by this undirected movement is considerably less than the change in larval distribution observed in the vertical behaviour chamber, suggesting that the latter movement is directed.

Several explanations may account for the directed movement, including positive buoyancy, response to a partial gradient of dissolved gasses, high barokinesis, orientation in (or response to) the earth's geomagnetic field, and response to a gravitational force. The larvae

of all three ascidian species are negatively buoyant (Chapter 7), so positive buoyancy is not a possible explanation. The water that the larvae were mixed in prior to introduction into the vertical behaviour chamber had been aerated and was therefore saturated with respect to atmospheric gasses; it seems unlikely that a partial gradient of dissolved gasses could develop in this water in the course of the one hour experiment.

The vertical behaviour chamber is one metre long, so that even without applied hydrostatic pressure there is one metre differential of water pressure between the top and the bottom of the chamber. However, pressure is a scalar quantity and can therefore only affect activity; a vector cue is required to produce directed motion. So barokinesis is not a suitable explanation.

No attempt was made to identify the influence of magnetic fields on the larvae because orientation to the earth's geomagnetic field appeared an unlikely explanation given that these species have a widespread latitudinal distribution, necessitating a variety of different angles of orientation to the field among populations; furthermore, in the extreme, populations would have had to have evolved oppositely directed responses in the northern and southern hemispheres. Orientation to gravity (a vector cue) appears to be the only suitable explanation.

Experiments will be described in later chapters that should further substantiate the claim that the negative geotactic response exhibited by these larvae is an active, direct, true gravity response. The response is greater for young larvae than for mature larvae. This enhanced response to may be an adaptation that quickly removes recently hatched larvae from the vicinity of benthic filter feeders which might engulf them.

CHAPTER 9 LARVAL RESPONSE TO LIGHT

9.1 Introduction

Light has been proposed as the principle ecological factor controlling the vertical distribution of planktonic animals (Spooner, 1933: Bainbridge, 1961; Thorson, 1964) but it is a highly variable parameter in the estuarine environment, its intensity and spectral composition varying more or less predictably as a function of season and time of day but much less predictably as a function of water quality and local weather conditions. The use of light as an orientation stimulus is not only complicated by its variable nature, but also by variables which may influence the organism's response such as previous exposure to light. The turbid conditions found in estuarine regions further complicate the situation by reducing the penetration of light into the water column by varying amounts; in addition to light intensity, wavelength and angular distribution vary with depth, and the scattering effect due to the small particles in the water column produces linear polarisation of light. The heterogeneous nature of estuarine turbidity can superimpose dramatic spatial and temporal variation in light penetration upon the existing variation in light intensity at a locus. Nevertheless, at a given depth, the downward ambient flux will be 100 times the upward flux (Clark & Denton, 1962); so in general, positive phototaxis produces orientation resulting in upward movement, negative phototaxis in downward movement.

The basic measurement of phototaxis is the movement of an individual organism toward or away from a defined light stimulus. However, variability among individuals in the direction and threshold of response is common, so that the sign of the response assigned to a population has to be based on an arbitrarily chosen proportional response level (Sulkin,

1984), i.e. when a response is described as being more positively (or negatively) phototactic, a larger percentage of a sample of organisms is responding accordingly. In functional terms, this means that the response threshold of a larger proportion of the sample population has been reached by experimental manipulation. The response is usually considered characteristic if greater than 30% of organisms tested respond in a given way (Sulkin, 1984).

Light flux (direction) and gravity are only physical cues in the aquatic environment which have vector properties and can be detected by larvae without a point of reference. It is therefore not surprising that the larvae of many marine benthic invertebrate species respond to light during their pelagic phase (Thorson, 1964). The simplest and the most universal response to light (and/or gravity) occurs at the first swimming stage when the emergent larvae swim upwards towards the light (Thorson, 1964); this response quickly removes them from the vicinity of benthic filter feeders which might engulf them. The larvae of many ascidian species are responsive to light flux during the free swimming period and at the time of settlement, in both the laboratory (Crisp & Ghobashy, 1971; Meadows & Cambell, 1972; Miller & Hadfield, 1986) and in the field (Olson, 1983).

Light intensity, like pressure, is a scalar quantity which can provide no orientation information to the larva unless its variation in space can be detected. Thus light intensity can only be used to determine orientation if it can be measured at several points and the direction of the light gradient established. Since light intensity increases as the larva approaches the source, flux and intensity usually act in tandem in the natural environment. However, the two cues generate different responses; light flux will produce taxis whereas light intensity may only initiate kinesis. The two cues must be separated if the responses to

them are to be examined in detail. It is relatively simple to demonstrate larval response to light flux, but much more careful experimentation is needed to reveal the response to light intensity; a gradient of light intensity at right angles to the direction of the incident light must be established (see Ryland, 1960). No attempt is made to separate the responses of pre-settlement larvae to light flux and light intensity in the present study; instead, the effect of variations in the intensity of light flux, as found in the natural environment, on the position held by larvae in the water column prior to settlement is examined.

Both light and gravity are usually directed vertically downwards from the surface so their individual effects are difficult to separate. Attempts have been made to isolate larval responses to light and gravity by reversing the direction of light whilst the direction of gravity remained constant (e.g. Sulkin, 1975). However, this only identifies the resultant response which is itself determined by the magnitude of the individual responses to light and gravity. An alternative experimental approach, which examines the responses of invertebrate larvae to light alone, employs a horizontally-orientated chamber with light shining along the axis from one side. Although an unnatural optical arrangement, this does eliminate the confounding effects of gravity (Forward & Costlow, 1974; Sulkin, 1975). Both approaches will be employed in this study, but the present chapter will concentrate on the responses of larvae in the horizontally-orientated chamber.

Many invertebrate larvae exhibit spectral sensitivity, i.e. they show a variation in response with the wavelength of applied light over a range of wavelengths (Forward & Cronin, 1979; Young, 1982). But as the results of the laboratory experiments are to be used to explain ascidian distribution, natural daylight was considered most relevant; use of this light source should facilitate the translation of the laboratory data to the field situation.

It is known that some ascidian larvae are influenced by a sudden variation in the level of illumination; sudden increases in light intensity cause the larvae to sink or to swim away from the light, sudden decreases cause them to swim upwards or towards the general direction of the light source (Young & Chia, 1985). Therefore great care was taken to avoid sudden changes in light intensity or any shadow falling on the experimental apparatus.

The problem of how to measure and present irradiance is controversial. The majority of studies involving light in the aquatic environment concern photosynthesis. Since photosynthesis is a photochemical process, the correct unit to use to measure irradiance in such studies is quanta per second per surface unit. If the illumination is considered in terms of energy, the units used may be watt m^{-2}, cal cm^{-2} (Langley) or $\mu E\, m^{-2}\, s^{-1}$. For more general ecological application, many investigators have chosen to measure light intensity in units of lux for light sources in the laboratory and daylight at the water surface. The lux unit does not take account of changes in the wavelength composition of the light, so its use is limited and it is now being phased out. However, for relative measurements at the water surface, or outside the experimental chamber, measurement of light intensity in units of lux is quite adequate, and suitable meters are cheap and readily available. Therefore light intensity has been measured in units of lux throughout this study.

9.2 Methods

The horizontal behaviour chamber (see section 5.6) was used in these experiments. The tube was aligned with the light source and the end-plate holes and the bottom holes were sealed with rubber bungs. The chamber was prepared and larvae introduced through the middle entry port as described in section 5.6. The chamber was left for one hour at constant

temperature, during which time the light level was checked every few minutes and adjusted as necessary. The tube was then rotated and the segments drained, with rinsing (10 μm filtered sea water) into labelled pots and preserved for later examination (section 5.2). The control experiments used are those reported in section 5.6.

All larvae were maintained under low light intensity conditions prior to experimentation (see section 5.1.4) so as to reduce the risk of habituation to the intensities employed in the experiments. As far as possible, light experiments were carried out during periods of constant natural light intensity. The light intensity was measured (digital luxmeter, Cat No. 610-815, RS Components Ltd) adjacent to the window of the horizontal chamber every few minutes. When necessary, fine adjustments to light intensity were made by adjusting venetian blinds well away from the behaviour chamber; no shadows or sudden changes in light intensity were produced in these measurements and adjustments. The light intensities tested were 0, 250, 500, 1000, 1500 and 2000 lux. The experiment was aborted if the light level varied more than ±10% from the target intensity. Larval responses to 0 lux light intensity are reported in Chapter 8.

It was not possible to carry out many experiments on young larvae. In the main spawning season *Ciona intestinalis* eggs hatched around midnight, so natural light could not be used in any experiments with young (<4 h old) larvae. Reliably young larvae of *Ascidiella aspersa* and *Styela clava* were only available soon after hatching commenced and, despite occurring around midday in late summer, light conditions were not always sufficiently constant to carry out experiments. Consequently the majority of light experiments involved mature larvae, with only occasional opportunistic experiments using young larvae.

9.3 Results

9.3.1 *Ciona intestinalis* larvae

Light at 250 lux intensity produced a significant ($p<0.005$, *G*-test) change in the distributions of larvae compared with those found in the controls (Table 42) and in the absence of light (Table 33). The mean percentage of larvae found in the section of the horizontal chamber furthest from the light source (window) was significantly greater ($p<0.01$, *t*-test) than that found in either control and in the absence of light, indicating negative phototaxis among a proportion of the population. The significance of the differences in mean percentage of mature *C. intestinalis* in the section of the chamber nearest the light source and the section furthest from the light source under different light intensities are summarised in Table 47.

Table 42 Distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber with 250 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	34 (4.7)	0 (0)	733
Expt. 1	188 (3.3)	978 (17.0)	2013 (34.9)	1256 (21.8)	1328 (23.0)	5763
Expt. 2	24 (3.0)	136 (16.9)	327 (40.6)	218 (27.0)	101 (12.5)	806
Expt. 3	25 (2.1)	193 (16.4)	468 (39.9)	310 (26.4)	178 (15.2)	1174
Young	40 (5.5)	222 (30.6)	284 (39.1)	99 (13.6)	81 (11.2)	726

Only one experiment was carried out with young larvae and this showed an increase in the proportion of larvae moving both towards and away from the light source.

The distributions of larvae at 500 lux light intensity were significantly different ($p<0.005$, *G*-test) to either control (Table 43) and those found in the absence of light (Table 33). The mean percentage of larvae found in the end sections of the horizontal chamber was not significantly different ($p>0.05$, *t*-test) from that found with 250 lux, indicating the continuation of negative phototaxis among a small proportion of the population (Table 47).

Table 43 Distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber with 500 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	34 (4.7)	0 (0)	733
Expt. 1	23 (1.9)	122 (10.0)	730 (59.9)	281 (23.1)	62 (5.1)	1218
Expt. 2	53 (6.1)	191 (22.1)	329 (38.1)	197 (22.8)	94 (10.9)	864
Expt. 3	56 (4.2)	393 (29.3)	505 (37.6)	184 (13.7)	204 (15.2)	1342
Young	103 (8.4)	327 (26.7)	493 (40.2)	129 (10.5)	174 (14.2)	1226

The one experiment carried out with young larvae showed a significant difference ($p<0.005$, *G*-test) in distribution compared with mature larvae, and an increase in the proportion of larvae moving towards and away from the light source compared with the distribution of young larvae at 250 lux.

The distributions of larvae observed at 1000 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 44) and those found in the absence of light (Table 33). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 0, 250 and 500 lux were applied; however, the mean percentage of

larvae found in the section of the chamber furthest from the light source was significantly greater ($p<0.05$, *t*-test) than that observed with 500 lux, indicating a continuation of negative phototaxis among a small proportion of the population (Table 47).

Table 44 Distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber with 1000 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	34 (4.7)	0 (0)	733
Expt. 1	13 (3.0)	75 (17.2)	136 (31.2)	75 (17.2)	137 (31.4)	436
Expt. 2	18 (2.7)	97 (14.6)	302 (45.6)	96 (14.5)	150 (22.6)	663
Expt. 3	49 (2.4)	233 (11.6)	458 (22.9)	509 (25.4)	752 (37.6)	2001
Young	311 (9.2)	1621 (47.7)	891 (26.2)	112 (3.3)	460 (13.5)	3395
Young	62 (7.1)	292 (33.2)	294 (33.4)	102 (11.6)	129 (14.7)	879
Young	36 (6.3)	152 (26.6)	219 (38.4)	73 (12.8)	91 (15.9)	571

Three experiments were carried out with young larvae; these larvae were produced very late in the spawning season. The distributions of these young larvae after exposure to 1000 lux light intensity were significantly different ($p<0.005$, *G*-test) from the distributions of young larvae observed after exposure to 0, 250 and 500 lux. The mean percentage of young larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0$ 05, *t*-test) at light intensity of 1000 lux to that observed when the experiment was carried out in darkness (Table 48); however, it was significantly greater ($p<0,01$, *t*-test) than that observed when mature larvae were exposed to 1000 lux light intensity. The mean percentage of larvae found in the section furthest from the light source was significantly

greater ($p<0.01$, *t*-test) than that observed when the experiment was carried out in darkness, and significantly less ($p<0.05$, *t*-test) than that observed for mature larvae exposed to 1000 lux light intensity (Table 48).

The distributions of larvae observed with light intensity of 1500 lux were significantly different ($p<0.005$, *G*-test) from either control (Table 45) and those found in the absence of light (Table 33). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 0, 250, 500 and 1000 lux were applied (Table 47), and the mean percentage of larvae found in the section furthest from the light source was not significantly different to that observed with 250 and 1000 lux light intensities.

Table 45 **Distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber with 1500 lux light intensity and no applied hydrostatic pressure**

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	34 (4.7)	0 (0)	733
Expt. 1	18 (9.4)	29 (15.1)	49 (25.5)	32 (16.7)	64 (33.3)	192
Expt. 2	18 (2.7)	97 (14.6)	302 (45.6)	96 (14.5)	150 (22.6)	663
Expt. 3	16 (1.7)	131 (13.5)	273 (28.2)	246 (25.4)	301 (31.1)	967
Young	134 (12.3)	461 (42.2)	286 (26.2)	161 (14.7)	50 (4.6)	1092
Young	33 (12.6)	91 (34.7)	104 (39.7)	23 (8.8)	11 (4.2)	262
Young	51 (11.5)	129 (29.0)	232 (52.1)	18 (4.0)	15 (3.4)	445

However, the mean percentage of larvae found in the section of the chamber furthest from the light source was significantly greater ($p<0.05$, *t*-test) than that observed with 0 and 500 lux (Table 47), indicating that negative phototaxis was still occurring among a small proportion of the population.

Three experiments were carried out with young larvae; these larvae were again produced very late in the spawning season. The distributions of these young larvae after exposure to light intensity of 1500 lux were significantly different ($p<0.005$, *G*-test) from the distributions of young larvae observed after exposure to 0, 250, 500 and 1000 lux. The mean percentage of young larvae found in the section of the horizontal chamber nearest the light source was significantly greater with 1500 lux light intensity than that observed when the experiment was carried out in darkness ($p<0.001$, *t*-test) and with 1000 lux ($p<0.05$, *t*-test) light intensity (Table 48); however, it was not significantly different ($p>0.05$, *t*-test) to that observed for mature larvae exposed to 1500 lux light intensity. The mean percentage of young larvae found in the section furthest from the light source was not significantly different ($p>0.05$, *t*-test) to that observed when the experiment was carried out in darkness (Table 48), but it was significantly less than that observed with 1000 lux light intensity ($p<0.001$, *t*-test) and when mature larvae were exposed to 1500 lux light intensity ($p<0.01$, *t*-test).

The distributions of mature larvae observed after exposure to light of 2000 lux intensity were significantly different ($p<0.005$, *G*-test) from all other distributions of mature larvae (Table 46), due to the increase in the proportion of larvae moving away from the light source. The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed at

all other light intensities; the mean percentage of larvae found in the section furthest from the light source was only significantly different ($p<0.05$, *t*-test) to that observed with 0, 250 and 500lux light intensity (Table 47), indicating an increased tendency to negative phototaxis among the population of larvae.

Table 46 Distribution (and %) of *C. intestinalis* larvae in the horizontal behaviour chamber with 2000 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	1 (0.1)	275 (37.5)	423 (57.7)	34 (4.7)	0 (0)	733
Expt. 1	22 (4.6)	29 (6.1)	51 (10.7)	65 (13.7)	308 (64.8)	475
Expt. 2	12 (3.1)	65 (16.9)	132 (34.4)	104 (27.1)	71 (18.5)	384
Expt. 3	25 (1.2)	75 (3.5)	307 (14.3)	591 (27.4)	1156 (53.7)	2154
Young	160 (16.6)	305 (31.7)	349 (36.3)	102 (10.6)	46 (4.8)	962
Young	44 (11.0)	167 (41.8)	133 (33.3)	36 (9.0)	20 (5.0)	400
Young	52 (17.3)	88 (29.2)	119 (39.5)	20 (6.6)	22 (7.3)	301

Three experiments were carried out with late-season young larvae. The distributions of these larvae after exposure to 2000 lux were significantly different ($p<0.005$, *G*-test) from the distributions of young larvae observed after exposure to 0, 250, 500 and 1000 lux, due to an increased proportion moving towards the light source. The mean percentage of young larvae found in the section of the horizontal chamber nearest the light source was significantly greater ($p<0.05$, *t*-test) at 2000 lux than that observed with 1000 lux light intensity (Table 48), but not significantly different ($p>0.05$, *t*-test) to that observed with 1500 lux light intensity. The mean percentage of young larvae found in the section nearest

the light source was also significantly greater ($p<0.01$, *t*-test) than that observed when the experiment was carried out with mature larvae. The mean percentage of young larvae found in the section furthest from the light source was significantly less ($p<0.01$, *t*-test) than that observed with 1000 lux (Table 48), but not significantly different ($p>0.05$, *t*-test) from that observed with 1500 lux. The mean percentage of young larvae found in the section furthest from the light source was significantly less ($p<0.05$, *t*-test) than that observed when the experiment was carried out with mature larvae.

Table 47 Significance of differences in mean percentages of mature *C. intestinalis* larvae in section A (nearest light) and section E (furthest from light) of the horizontal chamber under different light intensities

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500	2000	Dead control
0	-	**	ns	*	**	*	ns
250	ns	-	ns	ns	ns	*	**
500	ns	ns	-	*	*	**	*
1000	ns	ns	ns	-	ns	ns	**
1500	ns	ns	ns	ns	-	ns	**
2000	ns	ns	ns	ns	ns	-	*
Dead control	*	ns	ns	ns	ns	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Table 48 Significance of differences in mean percentage of young *C. intestinalis* larvae in section A (nearest light) and section E (furthest from light) of the horizontal chamber under different light intensities

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	1000	1500	2000	Dead control
0	-	**	ns	*	ns
1000	ns	-	***	**	***
1500	***	*	-	ns	ns
2000	ns	*	ns	-	*
Dead control	*	***	***	***	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

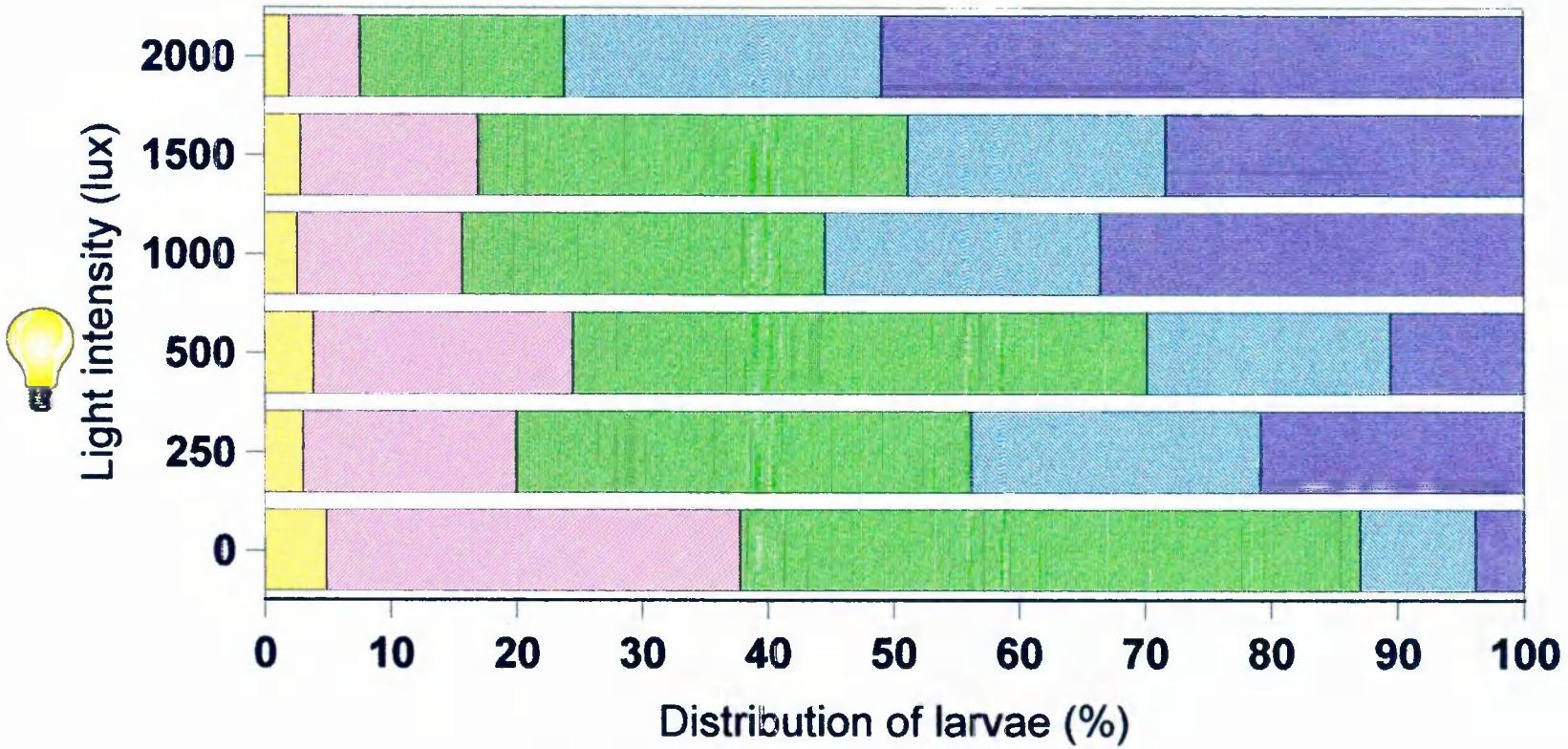
Analysis of variance of the results for mature larvae indicates that the differences in the mean percentages of larvae found in the section of the horizontal chamber nearest the light source are not significant (Table 49), but the differences in the mean percentages of mature larvae found in the section furthest from the light source are significant ($F_{5, 12} = 7.530$, $P<0.01$). The differences in the mean percentages of young larvae found in the section of the horizontal chamber nearest the light source are significant ($F_{3, 8} = 15.894$, $P<0.001$) and the differences in the mean percentages of young larvae found in the section furthest from the light source are also significant ($F_{3, 8} = 41.298$, $P<0.001$).

A priori analysis of variance was carried out to compare the mean percentages at either end of the horizontal behaviour chamber in the absence of light with the mean percentages in the same section after exposure to the five light intensities. This analysis indicated that there was no significant difference ($F_{1, 12} = 2.6922$) between the mean percentages of mature larvae observed in the section nearest the light source; but the mean percentages of young larvae found in this section after exposure to 1000, 1500 and 2000 lux were significant higher ($F_{1, 8} = 8.9243$, $p<0.05$) than the mean percentages found with no exposure to light.

Table 49 ANOVA summary table (*C. intestinalis* larvae)

Sample	Source of variation	SS	df	MS	F
Mature *C. intestinalis* larvae (light end of chamber)	Among groups	32.23071	5	6.446143	0.6753
	Within groups	114.5525	12	9.546041	
	Total	146.7832	17		
Mature *C. intestinalis* larvae (dark end of chamber)	Among groups	1865.349	5	373.0697	7.5297
	Within groups	594.5547	12	49.54622	
	Total	2459.903	17		
Young *C. intestinalis* larvae (light end of chamber)	Among groups	131.9048	3	43.96826	15.8939
	Within groups	22.13086	8	2.766357	
	Total	154.0356	11		
Young *C. intestinalis* larvae (dark end of chamber)	Among groups	265.4758	3	88.49194	41.2981
	Within groups	17.14209	8	2.142761	
	Total	282.6179	11		

Figure 16 Mean response of mature C. intestinalis larvae to light in the horizontal plane



See Figure 12 (page 95) for convention used in Figure 16.

In the section of the chamber furthest from the light source, the mean percentages of mature larvae found after exposure to light were significantly higher ($F_{1, 12} = 28.3924$, $p<0.001$) than the mean percentage found with no exposure to light. The mean percentages of young larvae found in the section furthest from the light source after exposure to light were not significantly different ($F_{1, 8} = 2.1109$) from the mean percentage found when no exposure to light occurred. Comparison of the mean percentages of young and old larvae found in the section of the horizontal behaviour chamber furthest from the light source indicated that, for light intensities of 1000, 1500 and 2000 lux, the mean percentage of mature larvae was significantly greater ($F_{1, 12} = 59.0623$, $p<0.001$) than that of young larvae.

There is no indication that the distributions change with larval density. The mean responses of mature *C. intestinalis* larvae to light in the horizontal plane are summarised graphically in Figure 16.

9.3.2 *Ascidiella aspersa* larvae

At a light intensity of 250 lux, the distribution of *A. aspersa* larvae in the horizontal behaviour chamber was significantly different ($p<0.005$, *G*-test) to both controls (Table 50) and to the distributions found in the absence of light (Table 37). The mean percentage of larvae found in the section nearest the light source was significantly greater ($p<0.05$, *t*-test) after exposure to 250 lux light intensity than when no exposure to light occurred. The mean percentage of larvae found in the section of the chamber furthest from the light source was significantly less ($p<0.05$, *t*-test) than that observed in the same section when the experiment was carried out in darkness. These changes in distribution indicate positive

phototaxis among a small proportion of the population. The significance of the differences in mean percentage of mature *A. aspersa* in the section of the chamber nearest the light source and the section furthest from the light source under different light intensities are summarised in Table 55.

Table 50 Distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber with 250 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Expt. 1	27 (6.5)	161 (38.5)	202 (48.3)	19 (4.5)	9 (2.2)	418
Expt. 2	21 (7.4)	108 (38.2)	131 (46.3)	18 (6.4)	5 (1.8)	283
Expt. 3	18 (6.0)	110 (36.5)	155 (51.5)	15 (5.0)	3 (1.0)	301

At 500 lux light intensity, the percentages of larvae found in the end sections of the horizontal chamber were similar to those found with 250 lux. The distributions were significantly different ($p<0.005$, *G*-test) to both controls (Table 51) and to the distributions found in the absence of light (Table 37). The mean percentage of larvae found in the section of the chamber nearest the light source was significantly greater ($p<0.05$, *t*-test) than that found in the same section in the absence of light (Table 55), indicating that positive phototaxis continued to be a response among a small proportion of the population. However, the mean proportion of larvae found in the section of the chamber furthest from the light source was not significantly different ($p>0.05$, *t*-test) to that found in the same section when the experiment was carried out in the absence of light, suggesting that the shift in distribution was due to movement of larvae in the middle sections. The one experiment

carried out with young larvae showed a significantly different ($p<0.05$, *G*-test) distribution to those of the mature larvae.

Table 51 Distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber with 500 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Expt. 1	44 (8.7)	199 (39.4)	219 (43.4)	31 (6.1)	12 (2.4)	505
Expt. 2	133 (6.6)	1154 (57.4)	660 (32.8)	32 (1.6)	32 (1.6)	2011
Expt. 3	16 (6.6)	73 (29.9)	118 (48.4)	29 (11.9)	8 (3.3)	244
Young	30 (9.2)	126 (38.7)	127 (39.0)	33 (10.1)	10 (3.1)	326

The distributions of larvae observed with 1000 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 52) and to the distribution found in the absence of light (Table 37). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 250 and 500 lux were applied (Table 55), but was significantly greater ($p<0.05$, *t*-test) to that found in the absence of light, indicating consistent negative phototaxis among a small proportion of the population. The mean percentage of larvae found in the section of the chamber furthest from the light source was not significantly different ($p>0.05$, *t*-test) from those observed at 0, 250 and 500 lux (Table 55).

Table 52 **Distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber with 1000 lux light intensity and no applied hydrostatic pressure**

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Expt. 1	78 (12.6)	230 (37.0)	215 (34.6)	50 (8.1)	48 (7.7)	621
Expt. 2	45 (9.8)	115 (25.2)	209 (45.7)	58 (12.7)	30 (6.6)	457
Expt. 3	20 (8.9)	45 (20.0)	123 (54.9)	34 (15.2)	2 (0.9)	224

The distributions of larvae observed at 1500 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 53) and to the distribution found in the absence of light (Table 37). The mean percentages of larvae found in both the section of the chamber nearest the light source and the section furthest from the light source were not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 0, 250, 500 and 1000 lux were applied (Table 55).

Table 53 **Distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber with 1500 lux light intensity and no applied hydrostatic pressure**

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Expt. 1	58 (4.3)	489 (36.1)	641 (47.3)	142 (10.5)	24 (1.8)	1354
Expt. 2	22 (6.7)	151 (45.8)	90 (27.3)	60 (18.2)	7 (2.1)	330
Expt. 3	33 (10.6)	108 (34.7)	120 (38.6)	29 (9.3)	21 (6.8)	311

The distributions of larvae observed at 2000 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 54) and the distribution found in the absence of light (Table 37). The mean percentages of larvae found in both the section of the chamber nearest the light source and the section furthest from the light source were not significantly different ($p>0.05$, *t*-test) to those observed at other light intensities (Table 55).

Table 54 Distribution (and %) of *A. aspersa* larvae in the horizontal behaviour chamber with 2000 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	3 (0.5)	207 (37.6)	321 (58.2)	19 (3.4)	1 (0.2)	551
Expt. 1	20 (7.1)	100 (35.7)	140 (50.0)	16 (5.7)	4 (1.4)	280
Expt. 2	18 (2.6)	107 (15.2)	285 (40.6)	204 (29.1)	88 (12.5)	702
Expt. 3	10 (5.1)	35 (17.8)	90 (45.7)	43 (21.8)	19 (9.6)	197

Table 55 Significance of differences in mean percentages of mature *A. aspersa* larvae in section A (nearest light) and section E (furthest from light) of the horizontal chamber under different light intensities

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500	2000	Dead control
0		*	ns	ns	ns	ns	ns
250	*	-	ns	ns	ns	ns	ns
500	*	ns	-	ns	ns	ns	ns
1000	*	ns	ns	-	ns	ns	ns
1500	ns	ns	ns	ns	-	ns	ns
2000	ns	ns	ns	ns	ns	-	ns
Dead control	ns	**	**	**	*	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

There is no indication that the distributions change with larval density.

Analysis of variance of the full results indicated that the differences in the mean percentages of larvae found in the section of the horizontal chamber nearest the light source are significant ($F_{5,12} = 4.3229$, $P<0.05$), but the differences in the mean percentages of larvae found in the section furthest from the light source are not significant (Table 56).

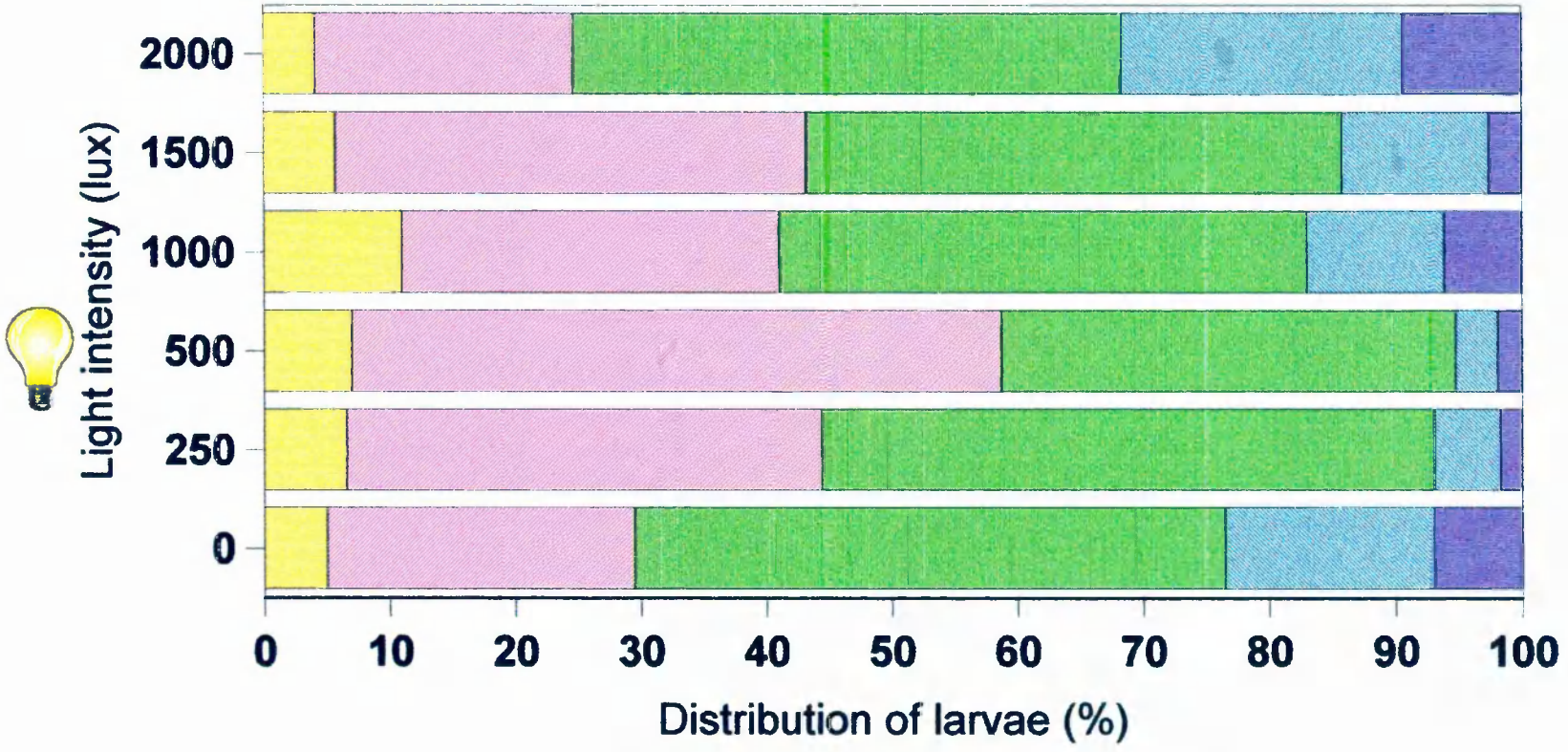
Table 56 ANOVA summary table (*A. aspersa* larvae)

Sample	Source of variation	SS	df	MS	F
Mature *A. aspersa* larvae (light end of chamber)	Among groups	111.1057	5	22.22114	4.32291
	Within groups	61.68384	12	5.14032	
	Total	172.7896	17		
Mature *A. aspersa* larvae (dark end of chamber)	Among groups	120.1609	5	24.03218	1.2772
	Within groups	225.7957	12	18.81631	
	Total	345.9565	17		

A priori analysis of variance was carried out to compare the mean percentages at either end of the horizontal behaviour chamber in the absence of light with the mean percentages in the same section after exposure to the five light intensities. This analysis indicated that there was no significant difference ($F_{1, 12} = 0.49730$) between the mean percentages of larvae observed in the section furthest from the light source; but the mean percentages of larvae found in the section of the chamber nearest the light source after exposure to light were significant higher ($F_{1, 12} = 10.11595$, $p<0.05$) than the mean percentage found with no exposure to light.

The mean responses of mature *A. aspersa* larvae to light in the horizontal plane are summarised graphically in Figure 17.

Figure 17 Mean response of mature A. aspersa larvae to light in the horizontal plane



See Figure 12 (page 95) for convention used in Figure 17.

9.3.3 *Styela clava* larvae

At a light intensity of 250 lux, the distribution of *S clava* larvae in the horizontal behaviour chamber was significantly different ($p<0.005$, *G*-test) to both controls (Table 57). After exposure to 250 lux light intensity, the mean percentages of larvae found in the section of the chamber nearest the light source and in the section furthest from the light source were not significantly different ($p>0.05$, *t*-test) to that found when no exposure to light occurred (Table 41). The significance of the differences in mean percentage of mature *S clava* in the section of the chamber nearest the light source and the section furthest from the light source under different light intensities are summarised in Table 62.

Table 57 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 250 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	4 (0.5)	308 (38.6)	456 (57.1)	30 (3.8)	1 (0.1)	799
Expt. 1	15 (3.9)	89 (23.1)	174 (45.1)	58 (15.0)	50 (13.0)	386
Expt. 2	10 (3.4)	84 (28.3)	141 (47.5)	40 (13.5)	22 (7.4)	297
Expt. 3	17 (2.8)	167 (27.2)	297 (48.4)	79 (12.9)	54 (8.8)	614

At 500 lux light intensity, the distributions of larvae were significantly different ($p<0.005$, *G*-test) to both controls (Table 58) and the distributions found in the absence of light (Table 41). The mean percentage of larvae found in the section of the horizontal chamber furthest from the light source was significantly greater ($p<0.05$, *t*-test) than that found in the same section when the experiment was carried out in the absence of light (Table 62), indicating a

negative phototactic response among a small proportion of the population. The mean proportion of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p<0.05$, *t*-test) to that found in the same section when the experiment was carried out in the absence of light, suggesting that the shift in distribution was due to movement of larvae in the middle sections.

Table 58 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 500 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	4 (0.5)	308 (38.6)	456 (57.1)	30 (3.8)	1 (0.1)	799
Expt. 1	36 (5.4)	121 (18.3)	277 (41.8)	116 (17.5)	112 (16.9)	662
Expt. 2	12 (3.9)	66 (21.6)	132 (43.1)	30 (9.8)	66 (21.6)	306
Expt. 3	41 (4.4)	185 (20.1)	356 (38.6)	154 (16.7)	186 (20.2)	922

The distributions of *S. clava* larvae observed at 1000 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 59) and the distributions found in the absence of light (Table 41). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 0, 250 and 500 lux were applied (Table 62). The mean percentage of larvae found in the section furthest from the light source was not significantly different ($p>0.05$, *t*-test) to that observed with 500 lux light intensity, but it was significantly greater ($p<0.05$, *t*-test) than that observed with no light and 250 lux, indicating continued negative phototaxis among a small proportion of the population. The results of

the one experiment carried out with young larvae suggested that a small proportion of the population of young larvae might be positively phototactic.

Table 59 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 1000 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	4 (0.5)	308 (38.6)	456 (57.1)	30 (3.8)	1 (0.1)	799
Expt. 1	24 (3.9)	128 (21.1)	148 (24.3)	160 (26.3)	148 (24.3)	608
Expt. 2	20 (4.8)	74 (17.7)	151 (36.2)	104 (24.9)	68 (16.3)	417
Expt. 3	31 (6.0)	83 (16.1)	204 (39.7)	116 (22.6)	80 (15.6)	514
Young	48 (13.4)	117 (32.8)	114 (31.9)	57 (16.0)	21 (5.9)	357

The distributions of *S. clava* larvae observed at 1500 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 60). and the distributions found in the absence of light (Table 41). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) to those observed when light intensities of 0, 250, 500 and 1000 lux were applied (Table 62). The mean percentage of larvae found in the section furthest from the light source was not significantly different ($p>0.05$, *t*-test) to that observed with light intensities of 250, 500 and 1000 lux, but it was significantly greater ($p<0.05$, *t*-test) than that observed with no light (Table 41), indicating continued negative phototaxis among a small proportion of the population.

Table 60 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 1500 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	4 (0.5)	308 (38.6)	456 (57.1)	30 (3.8)	1 (0.1)	799
Expt. 1	28 (6.0)	63 (13.4)	120 (25.6)	111 (23.7)	147 (31.3)	469
Expt. 2	24 (4.3)	60 (10.9)	294 (53.3)	90 (16.3)	84 (15.2)	552
Expt. 3	11 (6.4)	13 (7.6)	62 (36.2)	51 (29.8)	34 (19.9)	171

The distributions of larvae observed at 2000 lux light intensity were significantly different ($p<0.005$, *G*-test) from either control (Table 61) and the distributions found in the absence of light (Table 41). The mean percentage of larvae found in the section of the horizontal chamber nearest the light source was not significantly different ($p>0.05$, *t*-test) at 2000 lux to those observed with all other light intensities. The mean percentage of larvae found in the section furthest from the light source was significantly greater ($p<0.05$, *t*-test) than that observed with all other light intensities, suggesting that the threshold condition for a negative phototaxis response had been exceeded for a greater proportion of the population.

Table 61 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 2000 lux light intensity and no applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Dead control	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Mean live control	4 (0.5)	308 (38.6)	456 (57.1)	30 (3.8)	1 (0.1)	799
Expt. 1	266 (11.7)	220 (9.7)	362 (16.0)	593 (26.1)	828 (36.5)	2269
Expt. 2	4 (2.2)	6 (3.2)	25 (13.6)	44 (23.6)	106 (57.3)	185
Expt. 3	26 (7.2)	56 (15.5)	84 (23.3)	74 (20.5)	121 (33.5)	361

Table 62 Significance of differences in mean percentages of mature *S. clava* larvae in section A (nearest light) and section E (furthest from light) of the horizontal chamber under different light intensities

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500	2000	Dead control
0	-	ns	*	**	*	*	ns
250	ns	-	ns	*	ns	*	**
500	ns	ns	-	ns	ns	*	***
1000	ns	ns	ns	-	ns	*	**
1500	ns	ns	ns	ns	-	*	**
2000	ns	ns	ns	ns	ns	-	**
Dead control	ns	ns	ns	*	*	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

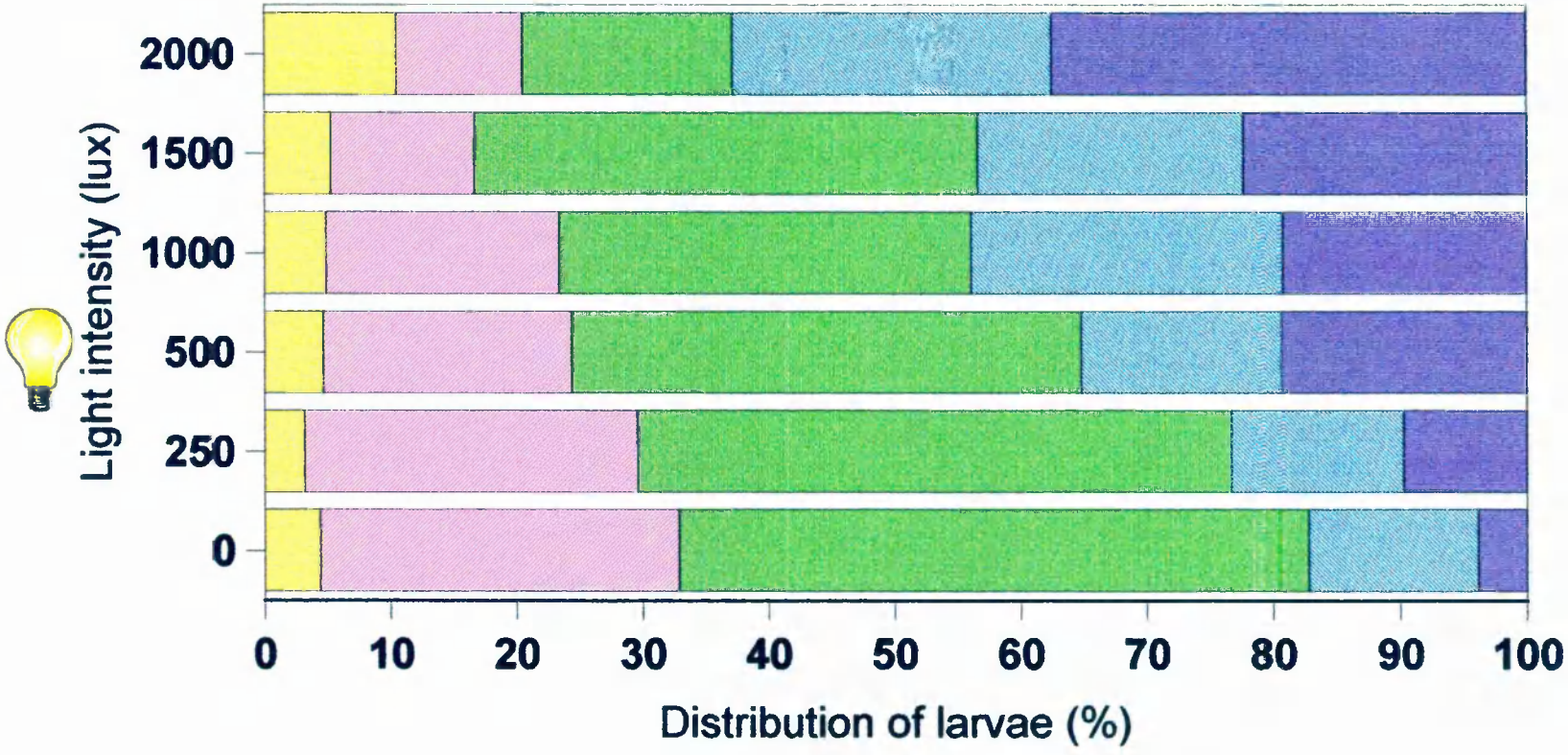
The mean responses of mature *S. clava* larvae to light in the horizontal plane are summarised in Figure 18, which shows clearly that the increase in the proportion of larvae in the section of the chamber furthest from the light source is achieved by movement of larvae from the middle sections. There is no indication that the distributions change with larval density.

Analysis of variance of the full results indicated that the differences in the mean percentages of larvae found in the section of the horizontal chamber nearest the light source are not significant ($F_{5, 12} = 4.3229$, $P<0.05$), but the differences in the mean percentages of larvae found in the section furthest from the light source are significant ($F_{5, 12} = 15.2716$, $P<0.010$). These results are presented in Table 63.

Table 63 ANOVA summary table (*S. clava* larvae)

Sample	Source of variation	SS	df	MS	F
Mature *S. clava* larvae (light end of chamber)	Among groups	29.4668	5	5.893359	0.89343
	Within groups	79.15625	12	6.596354	
	Total	108.623	17		
Mature *S. clava* larvae (dark end of chamber)	Among groups	1437.892	5	287.5783	15.2716
	Within groups	225.9707	12	18.83089	
	Total	1663.862	17		

Figure 18 Mean response of mature S. clava larvae to light in the horizontal plane



See Figure 12 (page 95) for convention used in Figure 18.

A priori analysis of variance was used to compare the mean percentages at either end of the horizontal behaviour chamber in darkness with the mean percentages in the same section after exposure to the five light intensities. This analysis indicated that there was no significant difference ($F_{1, 12} = 0.12926$) between the percentages of larvae observed in the section nearest the light source, but the mean percentages of larvae found in the section of the chamber furthest from the light source after exposure to light were significantly higher ($F_{1, 12} = 34.0292$, $p<0.001$) than the mean percentage found with no exposure to light.

9.4 Discussion

Studies of the response to light of aquatic larvae under experimental conditions are inevitably controversial, the majority of disagreement centring around the choice of light conditions used. Thorson (1964), in his review of light as a factor influencing the distribution of invertebrate larvae, concluded that no rules could be applied for determining the experimental light conditions to be employed because light penetration was so dependent on the local hydrographic conditions; choice of experimental intensities must be governed by the hydrographic conditions associated with the natural habitat of the species. The light intensities used in this study were chosen to span the range observed from the surface to 3.5 m depth in the Fawley intake channel on a relatively dull day. This range, 250-2000 lux is similar to that used by Bayne (1964) in his study of phototaxis in bivalve larvae.

When movement was restricted to the horizontal plane, mature *Ciona intestinalis* larvae exhibited negative phototaxis. The proportion of the population moving away from the light source generally increased with the applied light intensity, which suggests that the response

threshold of an increasing proportion of the population is exceeded as the light intensity is increased. However, the change in distribution could be due to high photokinesis, an increase in activity resulting from an increased intensity of light stimulus (Fraenkel and Gunn, 1961). The configuration of illumination employed in these experiments does not permit differentiation between a solely phototactic response and a phototactic response accompanied by high photokinesis. Nevertheless, it is possible to deduce the presence of a negative phototactic response since photokinesis alone would produce increased larval movement in all directions. Indeed, the two responses may be operating in tandem, offering the possibility of negative feedback control to maintain station at a specific light intensity which in turn could loosely correlate with depth in the water column.

The proportion of the mature *C. intestinalis* larval population exhibiting negative phototaxis at light intensities of between 1000 and 2000 lux exceeds 30%, thus this response can be considered characteristic. Since only mature larvae will be competent, these results will be directly relevant to larval selection of settlement sites.

At light intensities up to 1000 lux, young *C. intestinalis* larvae moved towards and away from the light source. The proportion of the young population sample moving away from the light source was less than for mature larvae and increased little with applied light intensity, reaching a maximum of 15% at 1000 lux. The variation in the proportion of the population moving away from the light source with increasing light intensity suggests that this is not a photokinetic response. Movement towards the light source generally increased with the applied light intensity up to 2000 lux, suggesting that the response threshold of an increasing proportion of the population was exceeded as the light intensity increased. However, the proportion of the population of young larvae exhibiting positive phototaxis

did not exceed 30%, so this response cannot be considered characteristic under the light conditions employed in these experiments. Nevertheless the results suggest that, when the response to light is isolated from that to gravity, a proportion of the population of *C. intestinalis* larvae undergoes an ontogenetic change in phototactic response.

A small proportion of mature *Ascidiella aspersa* larvae exhibited positive phototaxis when movement was restricted to the horizontal plane. The proportion of the population moving towards the light source did not vary with applied light intensity, but fluctuated between 5% and 10%. The lack of substantial change in the proportion of the population moving with respect to the light source when the light intensity was varied indicates that *A. aspersa* larvae do not respond photokinetically at these light intensities. The magnitude of the population shift is insufficient for it to be considered a characteristic phototactic response.

Mature larvae of *Styela. clava* exhibited negative phototaxis when movement was restricted to the horizontal plane. The proportion of the population moving away from the light source increased with applied light intensity, rising from approximately 10% of the population with a light intensity of 250 lux to over 40% of the population at 2000 lux light intensity. No significant increase was observed in the proportion of the population found nearest the light source. These results suggest that the response threshold for phototaxis of an increasing proportion of the population is exceeded as light intensity is increased, with or without high photokinesis, rather than an increasing proportion of the population exhibiting solely high photokinesis (the random movement resulting from high photokinesis alone would produce a more even distribution of the larval population throughout the chamber). The proportion of the population exhibiting negative phototaxis at 2000 lux light intensity exceeds 30%, thus this response can be considered characteristic.

PHYSICAL FACTORS INFLUENCING LARVAL BEHAVIOUR IN THREE SPECIES OF SOLITARY ASCIDIAN

VOLUME 2

M. H. DAVIS

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CHAPTER 10 LARVAL RESPONSE TO GRAVITY AND LIGHT IN COMBINATION

10.1 Introduction

The only physical cues in the aquatic environment with vector properties are light flux, gravity and water flow. Of these, only the first two can be detected by a larva without a point of reference. In the natural environment both of these cues operate in the same direction, i.e. vertically, making it difficult to attribute any response to an individual cue. I have attempted to identify the effect of individual cues by isolating the effect of gravity (Chapter 8) from that of light (Chapter 9). I will now attempt to assess the contributions of these cues when they operate in unison. This will be achieved by repeating the experiments to determine the effect of gravity (Chapter 8) with a variety of light intensities applied, as in the natural situation, in the opposite sense to gravity. The relative magnitude of the larval responses to the two cues will be assessed by a few experiments in which the direction of light flux is reversed so that the light cue reinforces the gravitational cue.

10.2 Methods

10.2.1 Light flux opposing gravity

The vertical behaviour chamber was deployed and operated as in section 8.2.1, except that the window fitting (see section 5.5) was attached to the top of the tube instead of a rubber bung being inserted into the end of the tube. The valve connecting the variable head tube and the chamber was closed and the tube filled with filtered (10 μm) sea water. A suspension of ascidian larvae (350 ml) was transferred to the behaviour chamber, the

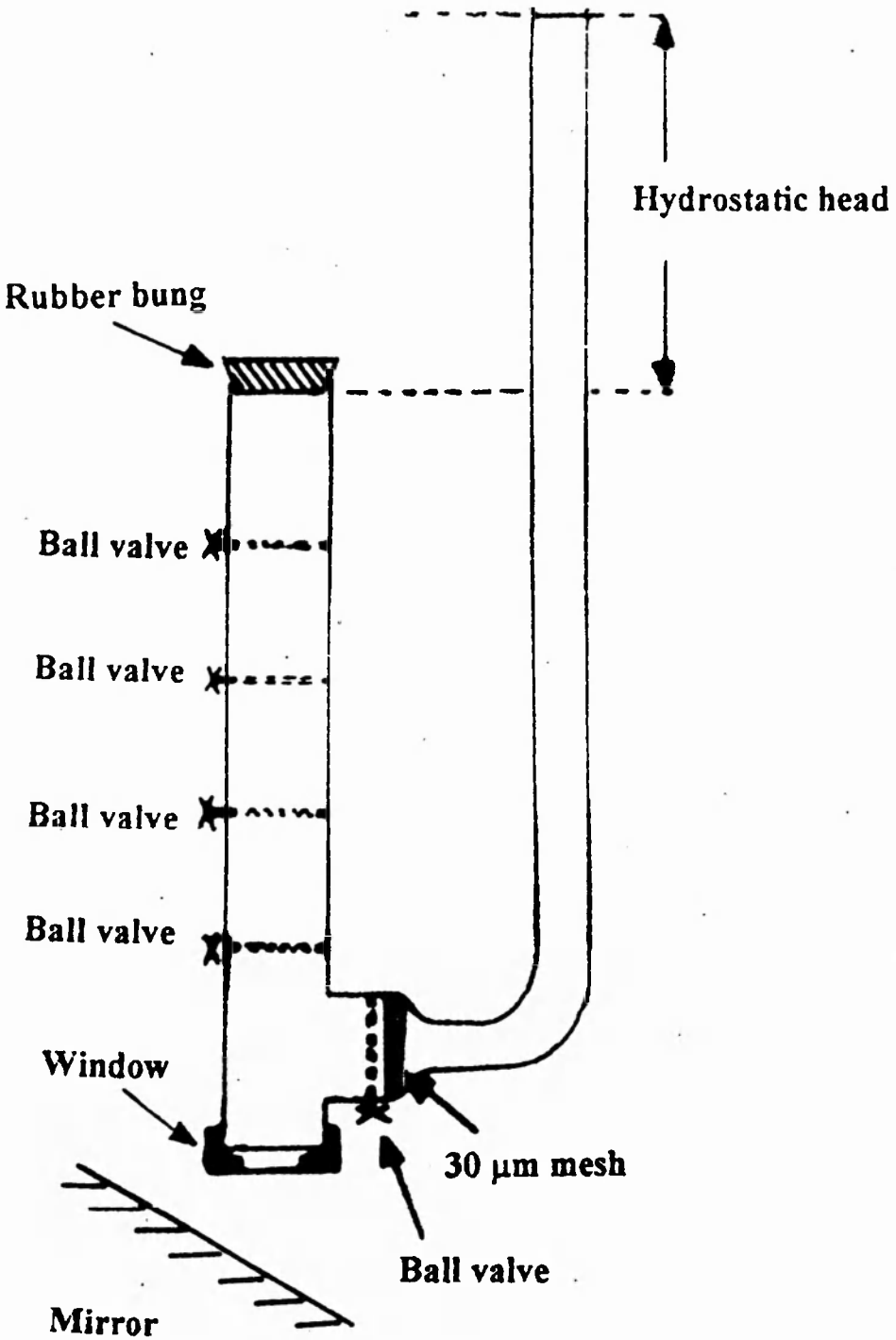
window attached to the top of the tube, the chamber inverted five times and fixed vertically. The main valve was opened and the height of variable head tube was adjusted to produce no hydrostatic head. The chamber was then left at constant temperature, with the light level monitored every few minutes, for one hour. The valves were then closed and the segments of water were decanted, with rinsing. Samples were stained and preserved for later examination (section 5.2). Light intensities of 250, 500, 1000 and 1500 lux were employed.

10.2.2 Light flux reinforcing gravity

For these experiments the bottom section of the tube in the vertical behaviour chamber was removed and a 70 mm tube inserted into the bottom valve. A ¾ inch Durapipe® ABS T-piece was attached to this pipe stub and another 70 mm length of pipe inserted into the T-piece to re-make the straight pipe. The window was fitted to the end of this section of pipe. Plankton netting (30 μm mesh) was glued to the end of a short stub of ¾ inch ABS pipe and the net end of the pipe was inserted into the side arm of the T-piece; the other end of this pipe stub was inserted into the valve which was connected to the transparent hose (Figure 19). This configuration allowed light to enter the bottom of the tube whilst still permitting the hydrostatic head to be varied. However, the volume of the bottom segment of the chamber was approximately 5% larger in these experiments owing to the dead space in the side arm of the T-piece, so the results do not correspond exactly with those described in the previous section (10.2.1).

In these experiments light was directed up the tube by a mirror placed just below the window. It was extremely difficult to measure and control the level of natural daylight entering the tube with this arrangement, so artificial light (60 W daylight bulb) was used.

Figure 19 **The configuration of the vertical behaviour chamber used to test the response of ascidian larvae to light flux reinforcing gravity.**



The valve connecting the variable head tube and the chamber was closed and the tube filled with filtered sea water (10 μm). A suspension of ascidian larvae (350 ml) was transferred to the chamber, the rubber bung inserted in the top of the chamber, the chamber inverted five times and fixed vertically. The side valve was opened and the height of variable head tube was adjusted to produce no hydrostatic head. The chamber was left at constant temperature for one hour, with the light level monitored every few minutes. After an hour, the valves were closed and the segments of water were decanted, with rinsing, from the top end of the chamber. The samples were stained and preserved for later examination (section 5.2).

A separate apparatus was not constructed for these experiment, so experiments with light reinforcing gravity could only be carried out by reconfiguring the apparatus used to test the response of light opposing gravity. The inconvenience of this reconfiguration, coupled with the problems of monitoring the light levels effectively, led to few of these experiments being carried out.

It was assumed that the control experiments carried out in Chapter 8 would apply equally to these experiments, irrespective of the configuration of the behaviour chamber.

10.3 Results

10.3.1 *Ciona intestinalis* larvae

The application of light at 250 lux intensity from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 64). However, the mean percentages of larvae in the top and bottom sections of the chamber were not significantly different ($p>0.05$, *t*-test) from those observed in the absence

of light. The significances of the differences in mean percentage of mature *C. intestinalis* in the top and bottom sections of the chamber under different light intensities are summarised in Table 68.

Table 64 Distribution (and %) of *C. intestinalis* larvae in the vertical behaviour chamber with 250 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 31)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	276 (23.8)	1611 (39.7)	1023 (36.8)	1028 (21.6)	458 (45.1)
Section B	244 (21.0)	536 (13.2)	391 (14.1)	902 (18.9)	123 (12.1)
Section C	191 (16.5)	750 (18.5)	308 (11.1)	829 (17.4)	48 (4.7)
Section D	155 (13.3)	429 (10.6)	303 (10.9)	728 (15.3)	79 (7.8)
Section E (bottom)	294 (25.4)	729 (18.0)	755 (27.2)	1282 (26.9)	307 (30.2)
Total number of larvae	1160	4055	2780	4769	1015

Only one experiment was carried out with light of 250 lux intensity applied from below; the distribution of larvae was significantly different ($p<0.005$, *G*-test) to that observed when light of the same intensity was applied from above.

Light at 500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 65). The mean percentage of larvae in the top section of the chamber was significantly less ($p<0.01$, *t*-test), and the mean percentage of larvae in the bottom section of the chamber was significantly greater ($p<0.01$, *t*-test), than that observed in the absence of light, indicating a movement of larvae away from the light.

Table 65 Distribution (and %) of *C. intestinalis* larvae in the vertical behaviour chamber with 500 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 31)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	276 (23.8)	41 (13.1)	197 (12.3)	681 (9.4)	1006 (44.2)
Section B	244 (21.0)	46 (14.7)	55 (3.4)	898 (12.4)	291 (12.8)
Section C	191 (16.5)	37 (11.9)	91 (5.7)	848 (11.7)	214 (9.4)
Section D	155 (13.3)	28 (9.0)	186 (11.6)	831 (11.5)	162 (7.1)
Section E (bottom)	294 (25.4)	160 (51.3)	1076 (67.0)	3996 (55.1)	603 (26.5)
Total number of larvae	1160	312	1605	7254	2276

In the one experiment carried out with light of 500 lux intensity applied from below, the distribution of larvae was significantly different ($p<0.005$, *G*-test) to that observed when light of the same intensity was applied from above; the percentage of larvae in the top section of the chamber was considerably greater, and that in the bottom section was considerably less, than that observed when light was applied from above.

Light at 1000 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 66). The mean percentage of larvae in the top section of the chamber was significantly greater ($p<0.05$, *t*-test) than that observed in the absence of light, but the mean percentage in the bottom section was not significantly different ($p>0.05$, *t*-test). This distribution of larvae suggests a weak movement towards the source of the light. However, a significantly different ($p<0.005$, *G*-test) distribution of larvae was observed when the direction of the light flux was changed to the bottom of the chamber, with a larger percentage of larvae accumulating in the top section of the chamber, furthest away from the light source.

Table 66 **Distribution (and %) of *C. intestinalis* larvae in the vertical behaviour chamber with 1000 lux incident light and in the absence of applied hydrostatic pressure**

	Mean 0 lux (Table 31)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	276 (23.8)	206 (39.9)	845 (32.0)	3429 (36.2)	406 (46.0)
Section B	244 (21.0)	70 (13.6)	314 (11.9)	1863 (19.6)	144 (16.3)
Section C	191 (16.5)	49 (9.5)	364 (13.8)	1509 (15.9)	86 (9.7)
Section D	155 (13.3)	50 (9.7)	302 (11.4)	983 (10.4)	63 (7.1)
Section E (bottom)	294 (25.4)	141 (27.3)	818 (30.9)	1699 (17.9)	184 (20.8)
Total number of larvae	1160	516	2643	9483	883

Light of 1500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 67). The mean percentage of larvae in the top section was significantly greater ($p<0.05$, *t*-test) than that observed in the absence of light, but that in the bottom section was not significantly different ($p>0.05$, *t*-test), suggesting movement towards the light source.

Table 67 **Distribution (and %) of *C. intestinalis* larvae in the vertical behaviour chamber with 1500 lux incident light and in the absence of applied hydrostatic pressure**

	Mean 0 lux (Table 31)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	276 (23.8)	3647 (56.7)	741 (45.6)	208 (44.4)	626 (41.6)
Section B	244 (21.0)	928 (14.4)	325 (20.0)	65 (13.9)	185 (12.3)
Section C	191 (16.5)	660 (10.3)	192 (11.8)	34 (7.3)	96 (6.4)
Section D	155 (13.3)	453 (7.0)	115 (7.1)	37 (7.9)	135 (9.0)
Section E (bottom)	294 (25.4)	745 (11.6)	252 (11.5)	124 (26.5)	464 (30.8)
Total number of larvae	1160	6433	1625	468	1506

A significantly different ($p<0.005$, *G*-test) distribution of larvae was observed when the light source was changed to the bottom of the chamber. However, when 1500 lux light intensity was applied from below, the percentage of larvae observed in the top section of the chamber was less than occurred with either 1000 lux applied from below or 1500 lux applied from above. The percentage of larvae that accumulated in the bottom section of the chamber with 1500 lux light intensity applied from below was greater than that observed when the light was applied from above and that found in the absence of light.

Table 68 Significance of differences in mean percentages of mature *C. intestinalis* larvae in section A (top) and section E (bottom) of the vertical chamber under different light intensities

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500
0	-	ns	**	ns	ns
250	ns	-	**	ns	ns
500	**	ns	-	**	**
1000	*	ns	***	-	ns
1500	*	ns	**	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

10.3.2 *Ascidiella aspersa* larvae

The application of light at 250 lux intensity from above produced a significant ($p<0.005$, *G*-test) change in the distribution of *A. aspersa* larvae compared with that found in the absence of light (Table 69). The mean percentage of larvae in the top section of the chamber was significantly greater ($p<0.01$, *t*-test), and that in the bottom section was significantly less ($p<0.01$, *t*-test), than observed in the absence of light. This distribution of larvae suggests a movement towards the source of the light. The significances of the differences in mean

percentage of mature *A. aspersa* in the top and bottom sections of the chamber under different light intensities are summarised in Table 73.

Only one experiment was carried out with light of 250 lux intensity applied from below; the distribution of larvae was significantly different ($p<0.005$, *G*-test) to that observed when light was applied from above. However, a similar percentage of larvae was found in the section of the chamber nearest the light source irrespective of the direction of light application, suggesting positive phototaxis.

Table 69 Distribution (and %) of *A. aspersa* larvae in the vertical behaviour chamber with 250 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 35)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	119 (19.3)	171 (45.4)	685 (51.8)	286 (50.7)	58 (24.4)
Section B	69 (11.3)	46 (12.2)	205 (15.5)	78 (13.8)	12 (5.0)
Section C	46 (9.5)	34 (9.0)	140 (10.6)	57 (10.1)	18 (7.6)
Section D	47 (9.6)	42 (11.1)	105 (7.9)	41 (7.3)	31 (13.0)
Section E (bottom)	245 (50.3)	84 (22.3)	187 (14.1)	102 (18.1)	119 (50.0)
Total number of larvae	613	377	1322	564	238

Light at 500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 70). However, the mean percentages of larvae in the top and bottom sections of the chamber were not significantly different ($p>0.05$, *t*-test) from those observed in the absence of light (Table 73).

In the one experiment carried out with light of 500 lux intensity applied from below, the distribution of larvae was significantly different ($p<0.005$, *G*-test) to that observed when the light was applied from above, and to that observed in the absence of light. The percentage of larvae in the bottom section of the behaviour chamber with light from below was considerably greater than that observed in the top section with light from above.

Table 70 Distribution (and %) of *A. aspersa* larvae in the vertical behaviour chamber with 500 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 35)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	119 (19.3)	172 (30.3)	58 (35.8)	321 (23.3)	150 (24.1)
Section B	69 (11.3)	93 (16.4)	24 (14.8)	205 (14.9)	36 (5.8)
Section C	46 (9.5)	80 (14.1)	28 (17.3)	165 (12.0)	57 (9.2)
Section D	47 (9.6)	73 (12.9)	22 (13.6)	120 (8.7)	83 (13.3)
Section E (bottom)	245 (50.3)	150 (26.4)	30 (18.5)	569 (41.2)	296 (47.6)
Total number of larvae	613	568	162	1380	622

Light at 1000 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 71). The mean percentage of larvae in the top section of the chamber was significantly greater ($p<0.05$, *t*-test), and that in the bottom section was significantly less ($p<0.05$, *t*-test), than that observed in the absence of light (Table 73). This distribution of larvae suggests a movement towards the source of the light.

When the direction of the light flux was changed to the bottom of the chamber, the distribution of larvae was significantly different ($p<0.005$, *G*-test) to that observed when light was applied from above, and a similarly large percentage of larvae accumulated in the of the section chamber nearest the light source (bottom section) suggesting positive phototaxis.

Table 71 Distribution (and %) of *A. aspersa* larvae in the vertical behaviour chamber with 1000 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 35)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	119 (19.3)	167 (31.1)	437 (54.5)	173 (35.5)	76 (22.6)
Section B	69 (11.3)	68 (12.7)	127 (15.8)	81 (16.6)	21 (6.2)
Section C	46 (9.5)	90 (16.8)	83 (10.3)	49 (10.0)	30 (8.9)
Section D	47 (9.6)	92 (17.1)	62 (7.7)	34 (7.0)	48 (14.2)
Section E (bottom)	245 (50.3)	120 (22.3)	93 (11.6)	151 (30.9)	162 (48.1)
Total number of larvae	613	537	802	488	337

Light at 1500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 72). The mean percentage of larvae in the top section of the chamber was significantly greater ($p<0.05$, *t*-test), and that in the bottom section was significantly less ($p<0.05$, *t*-test), than that observed in the absence of light (Table 73). This distribution of larvae suggests a movement towards the source of the light, similar to that observed with light of 1000 lux intensity. No experiments were carried out with light applied from below the behaviour chamber.

Table 72 **Distribution (and %) of *A. aspersa* larvae in the vertical behaviour chamber with 1500 lux incident light and in the absence of applied hydrostatic pressure**

	Mean 0 lux (Table 35)	Expt. 1	Expt. 2	Expt. 3
Section A (top)	119 (19.3)	173 (41.8)	578 (59.4)	193 (41.5)
Section B	69 (11.3)	70 (16.9)	186 (19.1)	73 (15.7)
Section C	46 (9.5)	73 (17.6)	83 (8.5)	56 (12.0)
Section D	47 (9.6)	54 (13.0)	52 (5.3)	43 (9.2)
Section E (bottom)	245 (50.3)	44 (10.6)	74 (7.6)	100 (21.5)
Total number of larvae	613	414	973	465

Table 73 **Significance of differences in mean percentages of mature *A. aspersa* larvae in section A (top) and section E (bottom) of the vertical chamber under different light intensities**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500
0	-	**	ns	*	*
250	**	-	ns	ns	ns
500	ns	*	-	**	**
1000	*	ns	***	-	ns
1500	*	ns	**	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

10.3.3 *Styela clava* larvae

The application of light at 250 lux intensity from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 74). The mean percentage of larvae in the top section of the chamber was significantly less ($p<0.01$, *t*-test), and that in the bottom section was significantly greater

($p<0.05$, *t*-test), than that observed in the absence of light. This distribution of larvae suggests a movement away from the source of the light. The significances of the differences in mean percentage of mature *S. clava* in the top and bottom sections of the chamber under different light intensities are summarised in Table 78.

Table 74 Distribution (and %) of *S. clava* larvae in the vertical behaviour chamber with 250 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 39)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	1274 (62.7)	26 (22.0)	712 (20.4)	339 (28.3)	809 (66.2)
Section B	418 (20.6)	22 (18.6)	368 (10.5)	163 (13.6)	173 (14.2)
Section C	138 (6.8)	22 (18.6)	413 (11.8)	160 (13.4)	117 (9.6)
Section D	88 (4.3)	11 (9.3)	403 (11.6)	161 (13.5)	72 (5.9)
Section E (bottom)	114 (5.6)	37 (31.4)	1593 (45.7)	374 (31.2)	51 (4.2)
Total number of larvae	2031	118	3489	1197	1222

Only one experiment was carried out with light of 250 lux intensity directed from below. With this flux configuration, the distribution of larvae was found to be significantly different ($p<0.005$, *G*-test) both to that observed when light was applied from above and in the absence of light. The largest percentage of larvae was found in the section of the chamber furthest from the light source irrespective of the direction of light application, suggesting negative phototaxis.

Light at 500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 75). However, the mean percentages of larvae in the top and bottom sections of the

chamber were not significantly different ($p>0.05$, *t*-test) from those observed in the absence of light (Table 78).

Table 75 Distribution (and %) of *S. clava* larvae in the vertical behaviour chamber with 500 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 39)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	1274 (62.7)	519 (45.8)	184 (44.1)	550 (48.4)	1301 (68.8)
Section B	418 (20.6)	137 (12.1)	61 (14.6)	214 (18.8)	259 (13.7)
Section C	138 (6.8)	117 (10.3)	45 (10.8)	128 (11.3)	168 (8.9)
Section D	88 (4.3)	76 (6.7)	55 (13.2)	76 (6.7)	102 (5.4)
Section E (bottom)	114 (5.6)	285 (25.1)	72 (17.3)	169 (14.9)	60 (3.2)
Total number of larvae	2031	1134	417	1137	1890

When light of 500 lux intensity was applied from below, the larval distribution was significantly different ($p<0.005$, *G*-test) to that observed with light applied from above and to that observed in the absence of light. With this configuration of light flux, the percentage of larvae in the top section of the chamber was considerably greater than that observed in the bottom section when light of the same intensity was applied from above.

Light at 1000 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 76). However, the mean percentages of larvae observed in both the top and bottom sections of the chamber were not significantly different ($p>0.05$, *t*-test) from those observed in the absence of light (Table 78).

Table 76 Distribution (and %) of *S. clava* larvae in the vertical behaviour chamber with 1000 lux incident light and in the absence of applied hydrostatic pressure

	Mean 0 lux (Table 39)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	1274 (62.7)	367 (51.5)	447 (67.5)	580 (56.1)	1094 (71.2)
Section B	418 (20.6)	126 (17.7)	89 (13.4)	115 (11.1)	183 (11.9)
Section C	138 (6.8)	61 (8.6)	39 (5.9)	57 (5.5)	130 (8.5)
Section D	88 (4.3)	47 (6.6)	22 (3.3)	118 (11.4)	86 (5.6)
Section E (bottom)	114 (5.6)	111 (15.6)	65 (9.8)	164 (15.9)	43 (2.8)
Total number of larvae	2031	712	662	1034	1536

When the direction of the light flux was changed to the bottom of the chamber, the distribution of larvae observed was significantly different ($p<0.005$, *G*-test) to that found when light was applied from above and to that found in the absence of light. The percentage of larvae that accumulated in the top section of the chamber, furthest from the light source, was larger than that found with light intensities of 250 and 500 lux, suggesting that, on the limited data available, the proportion of the population exhibiting negative phototaxis may increase with applied light intensity.

Light at 1500 lux intensity applied from above produced a significant ($p<0.005$, *G*-test) change in the distribution of larvae compared with that found in the absence of light (Table 77). The mean percentages of larvae in the top and bottom sections of the chamber were not significantly different ($p>0.05$, *t*-test) from that observed in the absence of light (Table 78).

Table 77 **Distribution (and %) of *S. clava* larvae in the vertical behaviour chamber with 1500 lux incident light and in the absence of applied hydrostatic pressure**

	Mean 0 lux (Table 39)	Expt. 1	Expt. 2	Expt. 3	Light direction reversed
Section A (top)	1274 (62.7)	374 (45.8)	184 (48.8)	278 (41.7)	571 (70.1)
Section B	418 (20.6)	115 (14.1)	64 (17.0)	138 (20.7)	94 (11.5)
Section C	138 (6.8)	86 (10.5)	39 (10.3)	92 (13.8)	60 (7.4)
Section D	88 (4.3)	62 (7.6)	27 (7.2)	67 (10.0)	61 (7.5)
Section E (bottom)	114 (5.6)	179 (21.9)	63 (16.7)	92 (13.8)	28 (3.4)
Total number of larvae	2031	816	337	667	814

In the one experiment carried out with direction of the light flux changed to below the chamber, the distribution of larvae was significantly different ($p<0.005$, *G*-test) to those observed when light was applied from above and in the absence of light. The percentage of larvae that accumulated in the top section of the chamber, furthest from the light source, was larger than that found with light intensities of 250 and 500 lux, indicating a continued trend towards negative phototaxis in the population.

Table 78 **Significance of differences in mean percentages of mature *S. clava* larvae in section A (top) and section E (bottom) of the vertical chamber under different light intensities**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light intensity (lux)	0	250	500	1000	1500
0	-	*	ns	ns	ns
250	**	-	*	*	*
500	ns	**	-	ns	ns
1000	ns	**	ns	-	ns
1500	ns	**	ns	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

10.4 Discussion

Although light at 250 lux intensity applied from above produced a significant change in the distribution of *C. intestinalis* larvae compared with that found in the absence of light, the percentages of larvae in the top and bottom sections of the chamber did not differ significantly in the two treatments. The same light intensity applied from below produced an increase in the percentage of larvae in both end sections of the chamber. The greatest increase was in the top section, furthest from the light; this appears to be due to negative phototaxis enhancing the negative geotactic larval response and, since larval movement exceeds negative buoyancy, this must be an active response.

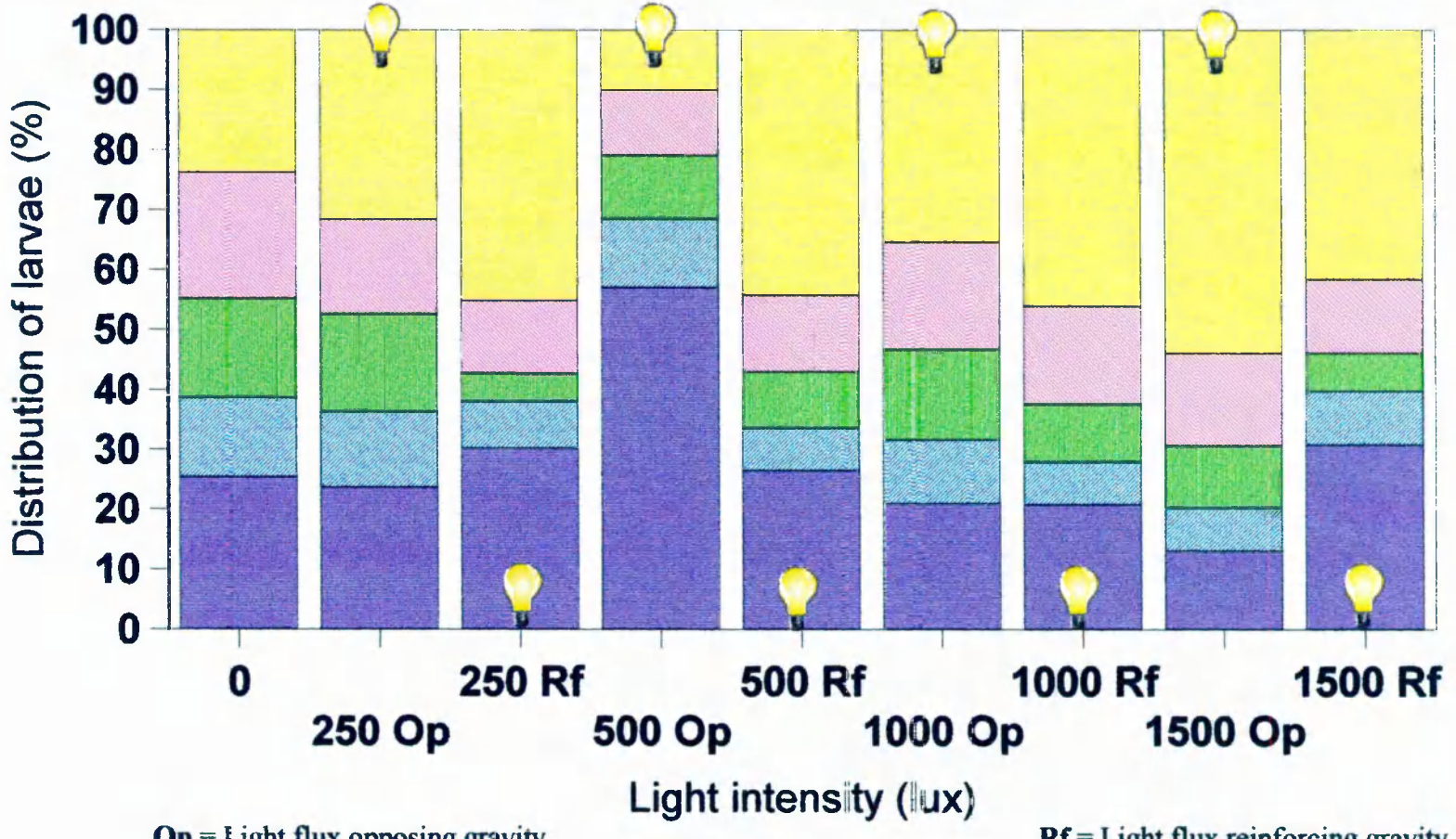
In contrast, when light at 500 lux intensity was applied from above the percentages of *C. intestinalis* larvae in the top and bottom sections of the chamber were significantly less and greater respectively than that observed in the absence of light. This redistribution of larvae indicates that they are moving away from the light, but offers no indication as to whether this is active negative phototaxis. The one experiment carried out with light from below produced a greater percentage of larvae in the top section of the chamber than when light was applied from above. Since this percentage was also greater than that found when the experiment was carried out in the absence of light, the larval response must be negative geotaxis supplemented by negative phototaxis. Furthermore, since the larvae would have to overcome negative buoyancy in order to congregate in the top section of the chamber, they must be actively moving away from the light.

When light at 1000 lux intensity was applied from above, the percentage of *C. intestinalis* larvae in the top section of the chamber was significantly greater than that found in the

absence of light, suggesting positive phototaxis. However, when the direction of light flux was inverted a larger percentage of larvae accumulated in the top section of the chamber, furthest from the light source. Thus a greater proportion of larvae than can be accounted for by negative geotaxis actively swim upwards irrespective of light direction, but this proportion increases when the light is from below. This suggests that at this light intensity the larval population exhibits ambivalent phototaxis, positive phototaxis when light is from above, but a greater negative phototaxic response when light is from below; both responses supplement the negative geotaxis observed in the absence of light. This may involve a change in the phototactic response of a portion of the larval population, or different responses from two portions of the population.

Light of 1500 lux intensity applied from above produced a significantly greater mean percentage of *C. intestinalis* larvae in the top, but not the bottom, section of the chamber than that observed in the absence of light. This distribution change suggests an active larval movement towards the light source which supplements negative geotaxis. When light was applied to the bottom of the chamber, the percentage of larvae that accumulated in the top section decreased and that in the bottom section increased. This suggests that a proportion of the larval population exhibit weak positive phototaxis at this light level which, like the response observed with 1000 lux light intensity, enhances negative geotaxis when light is applied from above, but another proportion of the population exhibit weak negative phototaxis that can only be detected when light is applied from below and it is reinforcing negative geotaxis. The increase in the percentage of larvae in the bottom section of the chamber when light was applied from below supports the hypothesis of two groups of larvae with opposite phototactic responses. The responses of *C. intestinalis* larvae to light and gravity are summarised in Figure 20.

Figure 20 Response of mature C. intestinalis larvae to light and gravity



Op = Light flux opposing gravity. **Rf** = Light flux reinforcing gravity.

See Figure 10 (page 88) for convention used in Figure 20.

Comparison of the larval distributions observed at the four light intensities with that found in the dark (Figure 20) suggests the presence of two groups of *C. intestinalis* larvae in the population with opposite phototactic responses; this is unlikely to be an ontogenetic effect (see section 9.4) as mature larvae were used throughout. With light applied from above, the population phototactic response changed from positive to negative at around 500 lux, changed back to positive by 1000 lux, then became increasingly positive as light intensity increased to 1500 lux. With light from below, the percentage of larvae in the top section of the chamber was approximately constant. The results suggest that for positive, but not negative, phototaxis the proportion of the population responding, or the response threshold, varies. However, the unverified "light from below" results must be treated with caution.

When light at 250 lux intensity was applied from above, the percentage of *Ascidiella aspersa* larvae in the top section of the chamber was significantly greater, and the percentage of larvae in the bottom section of the chamber was significantly less, than that observed in the absence of light, suggesting active movement of larvae towards the light source. The distribution with light flux from below was similar to that found in the absence of light. Approximately 50% of the larval population was found in the section of the chamber nearest the light source irrespective of the direction of light application, indicating positive phototaxis.

Light at 500 lux intensity applied from above produced a significant change in the distribution of *A. aspersa* larvae compared with that found in the absence of light, but the mean percentages of larvae in the top and bottom sections of the chamber did not differ significantly in the two treatments. Nevertheless, the percentages of larvae in the top section of the chamber were greater, and those in the bottom section were less, than those observed

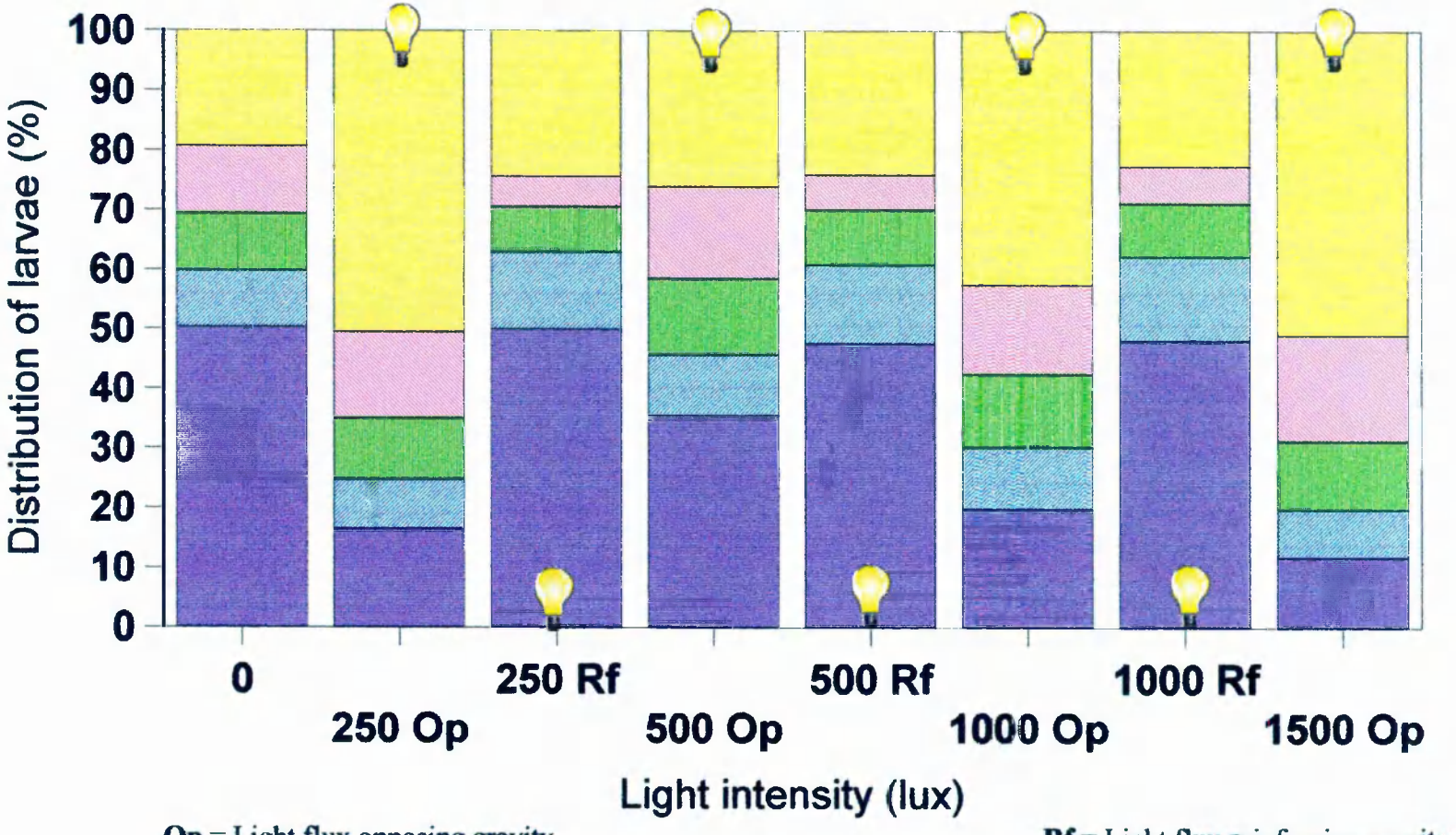
in the absence of light, suggesting active movement of larvae towards the light source. Light flux applied from below produced a distribution similar to that found in the absence of light, indicating the absence of a negative phototactic response in the population. The results support the suggestion of positive phototaxis at this light intensity.

Light of 1000 lux intensity applied from above produced a significantly greater percentage of *A. aspersa* larvae in the top section of the chamber, and a significantly smaller percentage in the bottom section, compared with that found in the absence of light. This change in larval distribution indicates a movement towards the source of the light. With light flux applied from below the chamber, the distribution was similar to that found in the absence of light, indicating the absence of negative phototaxis in the population of larvae.

Light at 1500 lux intensity applied from above produced a significantly greater percentage of *A. aspersa* larvae in the top section of the chamber, and a significantly smaller percentage of larvae in the bottom section, compared with that found in the absence of light. This distribution change indicates a movement towards the light source.

The responses of mature *A. aspersa* larvae to light and gravity are summarised in Figure 21. Comparison of the distributions observed at the four light intensities, with light from above, with that found in the absence of light suggests that the larvae are positively phototactic, with minimum response at around 500 lux. When light is applied from below, the distribution of larvae in the chamber is similar to that observed in the absence of light, indicating that there is no negative phototactic response in the population and that the positive phototactic response is weaker than the geotactic response. However, the "light from below" results are for single experiments and must therefore be treated with caution.

Figure 21 Response of mature A. aspersa larvae to light and gravity



Op = Light flux opposing gravity. **Rf** = Light flux reinforcing gravity.

See Figure 10 (page 88) for convention used in Figure 21.

When light at 250 lux intensity was applied from above, the percentage of *Styela clava* larvae in the top section of the chamber was significantly less, and the percentage of larvae in the bottom section of the chamber was significantly greater, than that observed in the absence of light, indicating movement of larvae away from the light source and a negative phototactic response strong enough to overcome the negative geotactic response. When light was applied from below, the largest percentage of larvae was found in the section furthest from the light source, but the distribution was similar to that found in the absence of light suggesting, in contrast, that the negative phototactic response is weak compared with the negative geotactic response.

Although light at 500 lux intensity applied from above produced a significant change in the distribution of *S. clava* larvae compared with that found in the absence of light, the mean percentages of larvae in the top and bottom sections of the chamber did not differ significantly in the two treatments. The percentages of larvae in the top section were less, and those in the bottom were greater, than were found with no light, indicating continued movement away from the light; but the response was less than for 250 lux light flux. Light from below produced a greater percentage of larvae in the top section of the chamber than light flux from above, but the light-below distribution was similar to the dark distribution, suggesting the phototactic response is weak compared with the geotactic response.

Light of 1000 lux intensity applied from above produced a significantly different larval distribution to that found with no light, but the mean percentages of larvae observed in both the top and bottom sections were not significantly different from those found in the absence of light. A significantly different larval distribution was observed with the light flux from below; a larger percentage of larvae accumulated in the top section of the chamber, furthest

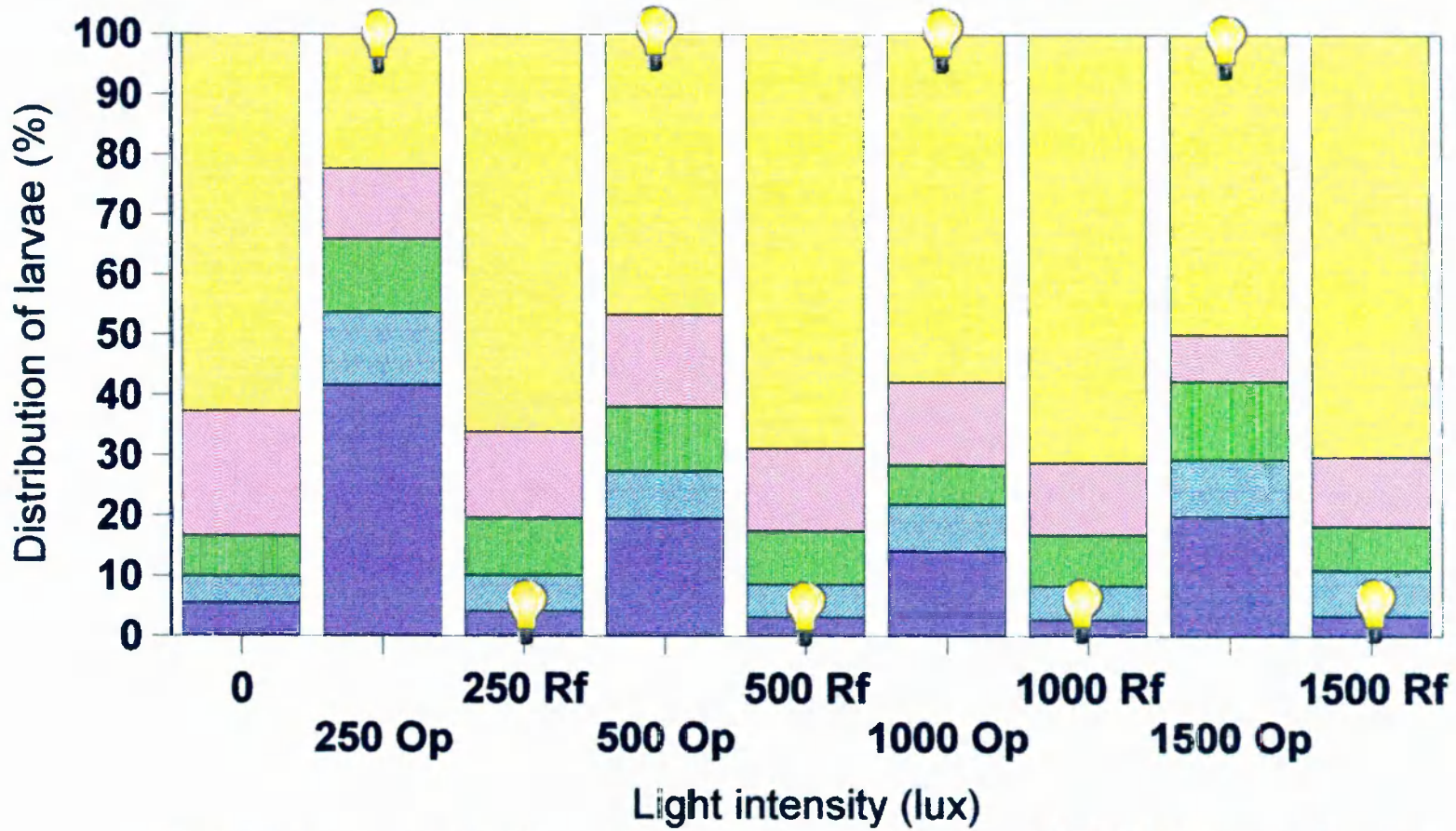
from the light source, than did with light from above, implying the phototactic response is weaker than the geotactic response.

Light at 1500 lux intensity applied from above produced a significant change in the distribution of *S. clava* larvae compared with that found in the absence of light, but the mean percentages of larvae in the top and bottom sections of the chamber were not significantly different from that observed in the absence of light. Nevertheless, the percentages of *S. clava* larvae in the top section were less, and the percentages of larvae in the bottom section were greater, than that observed in the absence of light, suggesting active movement of larvae away from the light source. The distribution was similar to that observed with light of 500 lux intensity applied from above. A significantly different larval distribution was observed when the direction of the light flux was changed to the bottom of the chamber; a larger percentage of larvae accumulated in the top section of the chamber, furthest from the light source, suggesting weak negative phototaxis.

Comparison of the distributions observed with light from above and in the absence of light suggests that the larvae are negatively phototactic, with maximum response at around 250 lux. With light from below, larval distribution is similar to that found in the absence of light, suggesting that negative phototaxis is a weak, possibly passive, response (but these results are for single experiments only).

The responses of mature *S. clava* larvae to light and gravity are summarised in Figure 22. Irrespective of the direction of light application, the largest percentage of larvae was found in the chamber section furthest from the light source indicating that any positive phototactic response present is weaker than the negative geotactic response.

Figure 22 Response of mature S. clava larvae to light and gravity



Op = Light flux opposing gravity. **Rf** = Light flux reinforcing gravity.

See Figure 10 (page 88) for convention used in Figure 22.

CHAPTER 11 LARVAL RESPONSE TO GRAVITY AND PRESSURE IN COMBINATION

11.1 Introduction

I have demonstrated (Chapter 8) that a substantial proportion of the larvae of all three species are negatively geotactic, swimming upwards in the absence of other cues; those experiments were carried out with no applied hydrostatic pressure, although the dimensions of the chamber enforced a 1 m head of water on larvae in the bottom section. Earlier (Chapter 7) I demonstrated that eggs and passive (anaesthetised) larvae were negatively buoyant and sank in still water. The inference must be that, in the natural environment, the eggs hatch at depth in the water column and the newly released larvae will be exposed immediately to some degree of positive hydrostatic pressure. The depth to which the eggs sink before hatching cannot easily be predicted; it will vary with physical factors such as the temperature and salinity, both of which affect the density of the water, and the degree of agitation. Nevertheless, it is obviously more realistic to examine the response of the larvae to gravity over a range of hydrostatic pressure.

The response to gravity and hydrostatic pressure will be determined by repeating the gravity only experiments (Chapter 8) with a variety of applied hydrostatic pressures, measured as metres head of water, ranging from 0.5 m to 3.5 m. Comparison with the effect of gravity in isolation (Chapter 8) should permit an assessment of the contribution of the response to hydrostatic pressure to the observed distribution when these two cues are operating in unison.

In the hatching beaker, young larvae (exposed to minimal hydrostatic pressure) swim up immediately after hatching then sink again, a cycle probably repeated many times before competence. The responses of mature, competent larvae are likely to be of most relevance in explaining pre-settlement behaviour, but the responses of young larvae may provide clues to initial dispersion behaviour. Therefore the responses of young, recently hatched larvae (< 2h old) and mature, near competent larvae (> 4h old) will be assessed separately.

11.2 Methods

The vertical behaviour chamber was deployed and operated as in section 8.2.1. The valve connecting the variable head tube and the chamber was closed and the tube filled with aerated filtered (10 μm) sea water. The behaviour chamber was filled to overflowing with a suspension of ascidian larvae in filtered (10 μm) sea water (approximately 350 ml), and the rubber bung was inserted in the top of the chamber in such a way as to avoid introducing any air. The chamber was inverted five times and was fixed vertically. The hydrostatic head was adjusted to the required level and the main valve was opened. The chamber was then left at constant temperature for one hour. A variety of hydrostatic pressures were applied by adjusting the height of the water level (hydrostatic head) in the polythene tube at the start of each experiment.

After an hour, the valves were closed and each segment of water was decanted in turn, with rinsing, from the top end of the chamber. The samples were stained and preserved for later examination (section 5.2). The control experiments described in section 8.1 were applied to the experiments described in the present section.

11.3 Results

11.3.1 Young *Ciona intestinalis* larvae

The distributions of young *C. intestinalis* larvae in the vertical behaviour chamber under applied hydrostatic pressures ranging from 0 to 3.5 m head of water, and in the absence of light, are presented in Table 79. The significances of the differences of the larval distributions (*G*-test) observed with the various hydrostatic pressures are presented in Table 80.

Young larvae tended to sink when applied hydrostatic pressure was increased from 0 to 2 m head of water (Figure 23). Further increases in hydrostatic pressure to 3.5 m head of water cause larvae to accumulate near the top of the chamber, a negative geotactic response that is active. The significances of differences in the mean percentages of young *C. intestinalis* in the top and bottom sections of the chamber under the range of hydrostatic pressures is presented in Table 81. The mean percentages in both sections when a pressure of 3.5 m head of water was applied were significantly different ($p<0.05$, *t*-test) from those observed at most other hydrostatic pressures. From the changes in larval distribution with hydrostatic pressure it would appear that, in darkness, a large proportion of larvae subjected to a pressure of 4.5 m depth (i.e. the bottom of the chamber) rise through the water column to around 2 m depth where many begin to sink again, forming a circulation cell in the water column. Hydrostatic pressures of less than 1 m head of water have little effect on larval distribution, so that larvae which are not drawn into the circulation cell and reach the surface layers in complete darkness will tend to remain at shallow depth.

Table 79 **Distribution (and %) of young *C. intestinalis* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																							
		0 (Table 30)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	177 (20.8)	395 (37.3)	1174 (33.4)	1454 (33.2)	443 (30.9)	1132 (24.3)	327 (28.0)	139 (26.2)	353 (19.3)	281 (17.2)	435 (18.3)	129 (24.9)	316 (16.0)	288 (14.8)	84 (16.7)	199 (13.3)	388 (18.5)	218 (16.3)	409 (23.5)	972 (34.5)	1475 (31.1)	1221 (41.7)	592 (52.7)	165 (47.0)	2052 (50.0)
Section B	150 (17.6)	140 (13.2)	625 (17.8)	662 (15.1)	161 (11.2)	1064 (22.8)	204 (17.5)	70 (13.2)	154 (8.4)	179 (10.9)	276 (11.6)	76 (14.7)	304 (15.4)	201 (10.3)	96 (19.0)	201 (13.4)	165 (9.0)	160 (12.0)	266 (15.3)	567 (20.1)	702 (14.8)	354 (12.1)	273 (24.3)	66 (18.8)	744 (18.1)
Section C	148 (17.4)	137 (12.9)	667 (19.0)	452 (10.3)	294 (20.5)	955 (20.5)	183 (15.7)	69 (13.0)	297 (16.2)	235 (14.4)	488 (20.5)	82 (15.8)	267 (13.5)	221 (11.4)	57 (11.3)	259 (17.3)	208 (11.4)	188 (14.1)	297 (17.1)	399 (14.2)	724 (15.3)	205 (7.0)	105 (9.3)	52 (14.8)	405 (9.9)
Section D	196 (23.0)	130 (12.3)	522 (14.9)	536 (12.2)	281 (19.6)	697 (14.9)	195 (16.7)	65 (12.3)	381 (20.8)	271 (16.6)	618 (26.0)	57 (11.0)	332 (16.8)	233 (12.0)	81 (16.1)	284 (19.0)	303 (16.6)	209 (15.7)	292 (16.8)	269 (9.5)	779 (16.4)	253 (8.6)	61 (5.4)	41 (11.7)	301 (7.3)
Section E (bottom)	182 (21.3)	258 (24.3)	522 (14.9)	1276 (29.1)	254 (17.7)	818 (17.5)	258 (22.1)	187 (35.3)	648 (35.4)	669 (40.9)	559 (23.5)	174 (33.6)	761 (38.4)	1004 (51.6)	186 (36.9)	555 (37.0)	814 (44.5)	559 (41.9)	474 (27.3)	612 (21.7)	1063 (22.4)	898 (30.6)	92 (8.2)	27 (7.7)	599 (14.6)
Number of larvae	853	1060	3510	4380	1433	4666	1167	530	1833	1635	2376	518	1980	1947	504	1498	1828	1334	1738	2819	4743	2931	1123	351	4101

Table 80 Significances of differences in distributions (*G*-test) of young *C. intestinalis* larvae with a variety of applied hydrostatic pressures in the absence of light

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*		*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*		*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*		*	*		*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

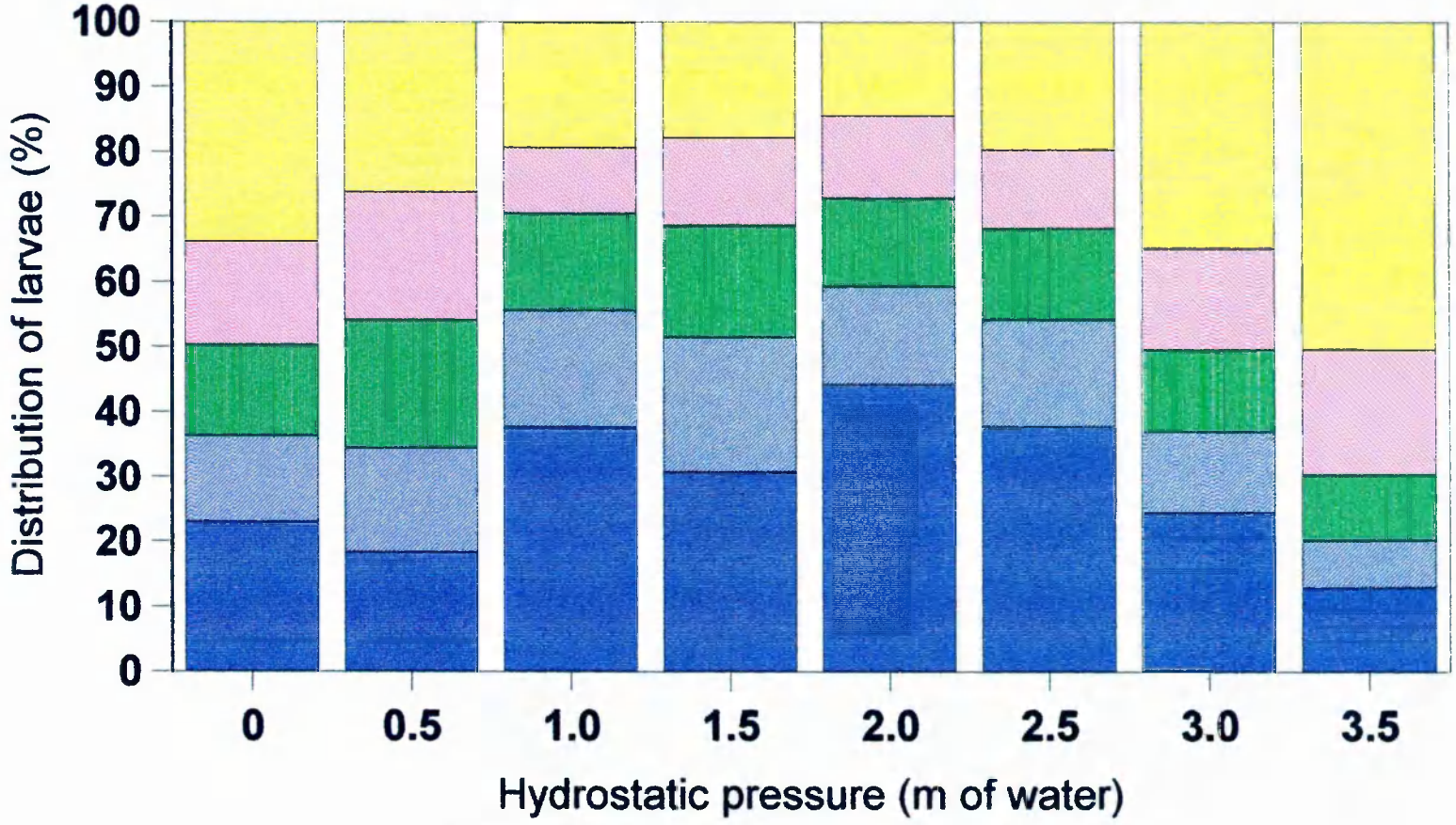
Table 81 Significances of differences in mean percentages (*t*-test) of young *C. intestinalis* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of hydrostatic pressures in the absence of light

(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	*	ns	ns	ns
0.5	ns	-	**	ns	*	ns	ns	*
1.0	*	ns	-	ns	ns	ns	*	**
1.5	*	ns	ns	-	ns	ns	ns	**
2.0	***	**	ns	ns	-	ns	ns	**
2.5	*	*	ns	ns	ns	-	ns	*
3.0	ns	ns	*	*	**	*	-	*
3.5	**	**	**	**	***	**	*	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Figure 23 Mean distributions of young C. intestinalis larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 23.

Table 82 **Distribution (and %) of mature *C. intestinalis* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																							
		0 (Table 31)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	177 (20.8)	221 (26.1)	405 (22.7)	201 (23.8)	557 (42.7)	57 (20.1)	1752 (33.9)	208 (30.0)	3538 (35.5)	237 (41.3)	203 (4.4)	98 (10.8)	276 (8.6)	3 (1.3)	123 (16.4)	195 (12.9)	144 (11.4)	162 (10.3)	158 (7.2)	304 (24.8)	172 (33.8)	1476 (29.7)	1804 (45.8)	1626 (67.3)	5479 (55.5)
Section B	150 (17.6)	242 (28.6)	281 (15.7)	208 (24.6)	265 (21.7)	47 (16.6)	928 (18.0)	108 (15.6)	1456 (14.6)	61 (10.6)	306 (6.6)	48 (5.3)	336 (10.4)	8 (3.5)	114 (15.2)	176 (11.6)	114 (9.0)	155 (8.3)	151 (6.9)	184 (15.0)	61 (12.0)	748 (15.1)	629 (16.0)	269 (10.5)	1462 (14.8)
Section C	148 (17.4)	127 (15.0)	330 (18.5)	117 (13.8)	196 (16.0)	46 (16.3)	701 (13.6)	132 (19.0)	1479 (14.9)	76 (13.2)	482 (10.3)	54 (6.0)	502 (15.6)	54 (23.9)	180 (23.9)	215 (14.2)	143 (11.3)	208 (11.1)	229 (10.4)	209 (17.0)	76 (14.9)	838 (16.9)	555 (14.1)	268 (10.5)	1111 (11.3)
Section D	196 (23.0)	118 (13.9)	235 (13.2)	111 (13.1)	102 (8.3)	70 (24.7)	623 (12.1)	117 (16.9)	1436 (14.4)	47 (8.2)	472 (10.1)	55 (6.1)	552 (17.1)	51 (22.6)	181 (24.1)	226 (15.0)	130 (10.3)	191 (10.2)	330 (15.0)	192 (15.6)	67 (13.1)	911 (18.3)	468 (11.9)	208 (8.2)	859 (8.7)
Section E (bottom)	182 (21.3)	139 (16.4)	536 (30.0)	208 (24.6)	82 (6.7)	63 (22.3)	1163 (22.5)	128 (18.5)	2044 (20.5)	153 (26.7)	3188 (68.5)	649 (71.8)	1562 (48.4)	110 (48.7)	154 (20.5)	699 (46.3)	731 (57.9)	1119 (60.0)	1332 (60.5)	339 (27.6)	133 (26.1)	994 (20.0)	480 (12.2)	181 (7.1)	964 (9.8)
Number of larvae	853	847	1787	845	1202	283	5167	693	9953	574	4651	904	3228	226	752	1511	1262	1835	2200	1228	509	4967	3936	2552	9875

11.3.2 Mature *Ciona intestinalis* larvae

The distributions of mature *C. intestinalis* larvae in the vertical chamber under applied hydrostatic pressure ranging from 0 to 3.5 m head of water, and in darkness, are presented in Table 82. Larval distributions at all hydrostatic pressures were significantly different ($p<0.05$, *G*-test) from each other. The mean distributions (Figure 24) show a tendency for mature larvae to sink at applied hydrostatic pressures between 1.5 m and 2.5 m head of water. At applied hydrostatic pressures less than 1.5 m and greater than 2.5 m head of water, larvae tend to accumulate near the top of the chamber. The significances of differences in mean percentages of mature *C. intestinalis* larvae in the top and bottom sections of the chamber are presented in Table 83.

Table 83 Significances of differences in mean percentages (*t*-test) of mature *C. intestinalis* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of hydrostatic pressures in the absence of light

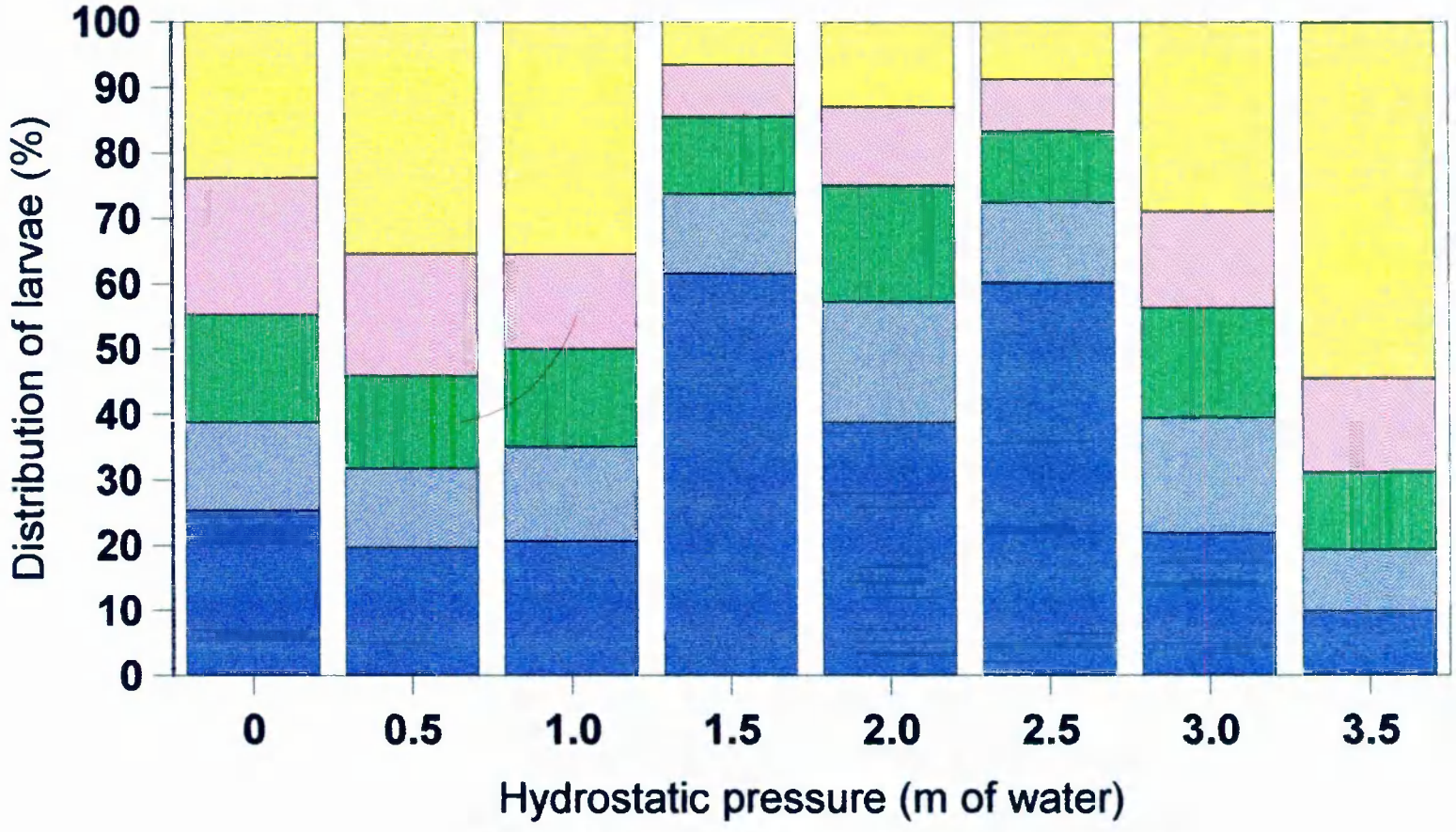
(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	*	ns	*	ns	*
0.5	ns	-	ns	**	ns	*	ns	ns
1.0	ns	ns	-	*	ns	**	ns	*
1.5	*	*	**	-	ns	ns	*	*
2.0	ns	ns	*	ns	-	ns	ns	ns
2.5	**	ns	**	ns	ns	-	**	**
3.0	ns	ns	ns	**	ns	**	-	**
3.5	*	ns	ns	**	**	*	*	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

In general, the response of mature larvae in darkness is similar to that of young larvae, with a circulation cell formed below 2.5 m in the water column, and hydrostatic pressures of less than 1 m head of water having little effect on distribution.

Figure 24 Mean distributions of mature C. intestinalis larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 24.

Analysis of variance indicated that there were significant differences in the mean percentages of *C. intestinalis* larvae found in the top ($F_{15, 32}$ = 14.86359, $p<0.001$) and bottom ($F_{15, 32}$ = 12.22833, $p<0.001$) sections of the vertical chamber (Table 84). Separate analysis of the data for young and mature larvae revealed significant differences in the mean percentages of young larvae found in the top ($F_{7, 16}$ = 26.20552, $p<0.001$) and the bottom ($F_{7, 16}$ = 9.685138, $p<0.001$) sections, and significant differences in the mean percentages of mature larvae found in the top ($F_{7, 16}$ = 13.45911, $p<0.001$) and the bottom ($F_{7, 16}$ = 13.73633, $p<0.001$) sections (Table 84).

Table 84 ANOVA summary table

Sample	Source of variation	SS	df	MS	F
Young & mature *C. intestinalis* (Top section of chamber)	Among groups	3969.977	15	264.6651	14.86359
	Within groups	569.8008	32	17.80627	
	Total	4539.777	47		
Young & mature *C. intestinalis*(Bottom section of chamber)	Among groups	4411.535	15	294.1024	12.22833
	Within groups	769.6289	32	24.0509	
	Total	5181.164	47		
Young *C. intestinalis* (Top section of chamber)	Among groups	1143.856	7	163.4080	26.20552
	Within groups	99.77013	16	6.235633	
	Total	1243.626	23		
Young *C. intestinalis* (Bottom section of chamber)	Among groups	1133.541	7	161.9344	9.685138
	Within groups	267.5181	16	16.71988	
	Total	1401.059	23		
Mature *C. intestinalis* (Top section of chamber)	Among groups	2767.687	7	395.3839	13.45911
	Within groups	470.0269	16	29.37668	
	Total	3237.714	23		
Mature *C. intestinalis* (Bottom section of chamber)	Among groups	3098.844	7	442.6919	13.73633
	Within groups	515.6451	16	32.22782	
	Total	3614.489	23		

A priori analysis of variance to compare the mean percentages of young and mature larvae in the top and bottom sections of the chamber over the full range of hydrostatic pressures, in the absence of light, indicated that there was no significant difference between the mean percentages of young and mature larvae found in the top section ($F_{1, 32}$ = 3.281573) or bottom section ($F_{1, 32}$ = 2.188122) of the vertical chamber.

11.3.3 Young *Ascidiella aspersa* larvae

The distributions of young *A. aspersa* larvae in the vertical behaviour chamber under applied hydrostatic pressures ranging from 0 to 3.5 m head of water, and in the absence of light, are presented in Table 85. The significances of the differences of larval distributions (*G*-test) observed with the various hydrostatic pressures are presented in Table 86.

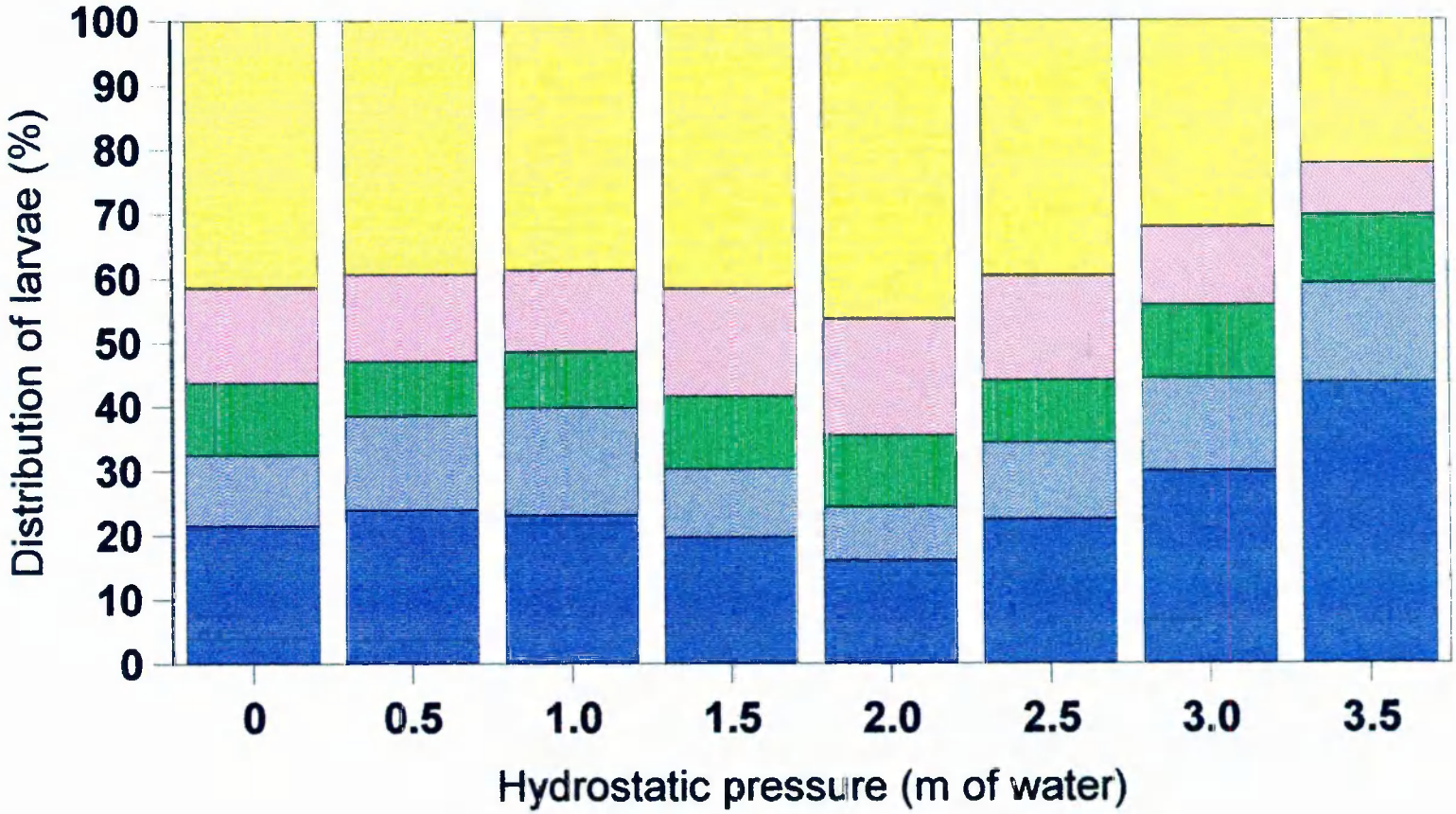
Apart from a slight increase in the population negative geotactic response at 2 m hydrostatic pressure, there was little difference in the mean larval distributions between 0 m and 2.5 m head of water; approximately 40% of young larvae were found in the top section of the chamber and 20% were found in the bottom section (Figure 25), indicating that the negative geotactic response of the larvae is not affected by these pressure increases.

At hydrostatic pressures greater than 2.5 m young larvae exhibit a tendency to sink, with approximately 20% of young larvae found in the top section and 40% found in the bottom section of the chamber at 3.5 m head of water. In fact, the mean percentages of larvae in the top and bottom sections of the chamber with 3.5 m hydrostatic pressure are significantly different ($p<0.05$, *t*-test) from the percentages found at all other applied pressures less than 3.0 m head of water (Table 87). This tendency to sink could result from a change in the direction of the geotactic response of a proportion of the population, a change from high barokinesis to low barokinesis or the cessation of swimming activity. A change in the direction of geotaxis would produce active migration, whereas a change in barokinesis or cessation of swimming would produce passive migration (resulting from the negative buoyancy of the larvae).

Table 85 **Distribution (and %) of young *A. aspersa* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																						
		0 (Table 34)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	153 (18.1)	438 (43.6)	188 (36.6)	183 (42.1)	187 (38.3)	253 (40.7)	166 (38.9)	319 (37.6)	89 (38.9)	95 (44.0)	149 (44.2)	173 (39.6)	129 (41.5)	159 (45.2)	125 (45.6)	339 (47.2)	167 (42.2)	351 (38.9)	150 (38.8)	134 (30.0)	160 (30.2)	164 (36.3)	86 (20.9)	163 (29.9)	170 (18.6)
Section B	194 (22.9)	109 (10.8)	106 (20.6)	76 (17.5)	66 (13.5)	92 (14.8)	49 (11.5)	102 (12.0)	34 (14.6)	28 (13.0)	48 (14.2)	80 (18.3)	54 (17.4)	63 (17.9)	49 (17.9)	131 (18.2)	66 (16.7)	137 (15.2)	73 (18.9)	48 (10.8)	45 (8.5)	81 (17.9)	30 (7.3)	32 (5.9)	87 (9.5)
Section C	198 (23.4)	125 (12.4)	48 (9.3)	47 (10.8)	40 (8.2)	48 (7.7)	44 (10.3)	84 (9.9)	19 (8.2)	11 (5.1)	33 (9.8)	55 (12.6)	36 (11.6)	42 (11.9)	36 (13.1)	73 (10.2)	36 (9.1)	92 (10.2)	36 (9.3)	54 (12.1)	55 (10.4)	54 (11.9)	43 (10.5)	46 (8.4)	109 (11.9)
Section D	147 (17.4)	129 (12.8)	43 (8.4)	41 (9.4)	85 (17.4)	78 (12.5)	62 (14.5)	142 (16.7)	42 (18.0)	33 (15.3)	39 (11.6)	44 (10.1)	31 (10.0)	31 (8.8)	19 (6.9)	60 (8.4)	51 (12.9)	119 (13.2)	30 (7.8)	75 (16.8)	86 (16.3)	44 (9.7)	62 (15.1)	81 (14.8)	147 (16.0)
Section E (bottom)	154 (18.2)	204 (20.3)	129 (25.1)	88 (20.2)	110 (22.5)	151 (24.3)	106 (24.8)	201 (23.7)	49 (21.0)	49 (22.7)	68 (20.2)	85 (19.5)	61 (19.6)	57 (16.2)	45 (16.4)	115 (16.0)	76 (19.2)	204 (22.6)	98 (25.3)	135 (30.3)	183 (34.6)	109 (24.1)	190 (46.2)	22.4 (41.0)	403 (44.0)
Number of larvae	846	1005	514	435	488	622	427	848	233	216	337	437	311	352	274	718	396	903	387	446	529	452	411	546	916

Figure 25 Mean distributions of young A. aspersa larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 25.

Table 86 Significances of differences in distributions (*G*-test) of young *A. aspersa* larvae with a variety of hydrostatic pressures in the absence of light

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3		*	*			-																		
1.0	1	*	*	*		*		-																	
	2	*	*	*					-																
	3	*	*	*						-															
1.5	1		*		*		*	*	*	*	-														
	2	*	*		*	*	*	*	*	*	*	-													
	3	*	*		*	*	*	*	*	*			-												
2.0	1	*	*		*	*	*	*	*	*	*			-											
	2	*	*		*	*	*	*	*	*	*				-										
	3	*	*	*	*	*	*	*	*	*	*	*	*			-									
2.5	1	*	*		*		*	*	*	*				*	*	*	-								
	2	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	-							
	3	*			*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		-				
	3	*			*	*	*	*	*	*	*			*	*	*	*			*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05;

Table 87 Significances of differences in mean percentages (*t*-test) of young *A. aspersa* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of hydrostatic pressures in the absence of light

(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

Applied hydrostatic pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	***
0.5	ns	-	ns	*	**	ns	ns	**
1.0	ns	ns	-	ns	*	ns	ns	**
1.5	ns	ns	ns	-	***	ns	ns	**
2.0	ns	**	ns	ns	-	ns	*	**
2.5	ns	ns	ns	ns	*	-	ns	***
3.0	*	ns	*	*	*	*	-	*
3.5	*	*	*	*	*	*	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

11.3.4 Mature *Ascidiella aspersa* larvae

The distributions of mature *A. aspersa* larvae in the vertical behaviour chamber under hydrostatic pressures ranging from 0 to 3.5 m head of water, and in the absence of light, are presented in Table 88. More of the larval distributions are significantly different ($p<0.05$, *G*-test) than for young larvae (Table 89).

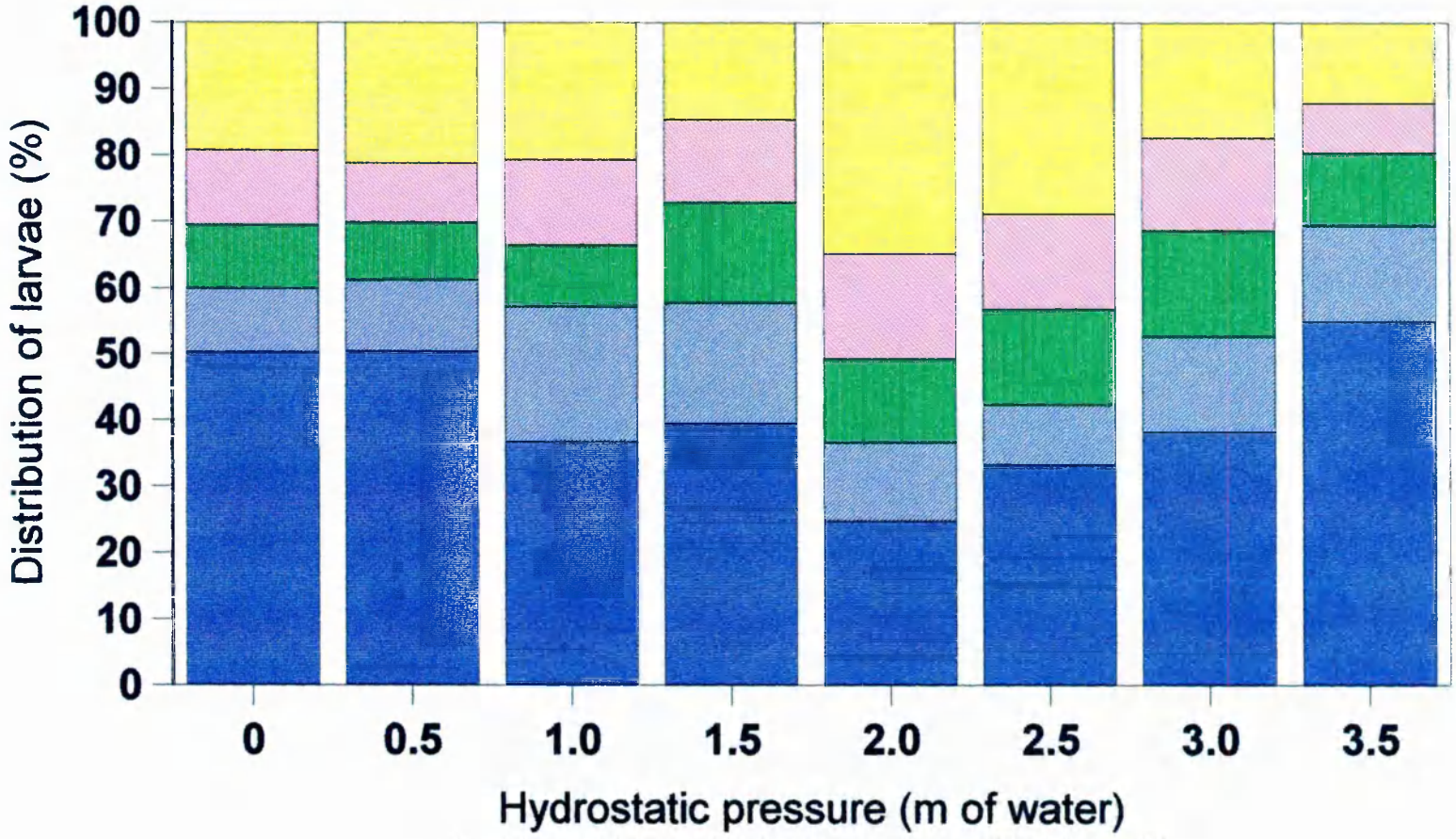
Mean larval distributions show a decline in the proportion of mature larvae in the bottom section of the chamber between 0 m and 2.0 m head of water, with a commensurate increase in the proportion of larvae in the middle or top sections (Figure 26). As with the young *A. aspersa* larvae, the maximum negative geotactic response was observed at 2.0 m head of water, but mature larval segregation was less pronounced with approximately 35% of larvae found in the top section of the chamber and 25% in the bottom section.

At hydrostatic pressures greater than 2.0 m mature larvae exhibit a tendency to sink, with only approximately 12% of the mature larvae found in the top section of the chamber at 3.5 m head of water but 55% of the larvae found in the bottom section of the chamber. Although the mean percentages of larvae in the top and bottom sections of the chamber with 3.5 m hydrostatic pressure were respectively less and greater than those recorded with young larvae, the values were not significantly different ($p>0.05$, *t*-test). Nor were the mean percentages of larvae in the top and bottom sections of the chamber with 3.5 m hydrostatic pressure significantly different ($p>0.05$, *t*-test) from the percentages of mature larvae found at all other applied pressures (Table 90). Nevertheless, there appears to be a trend towards larvae sinking with increasing hydrostatic pressure.

Table 88 **Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																						
		0 (Table 35)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	153 (18.1)	288 (22.5)	32 (17.9)	36 (9.4)	38 (20.8)	23 (7.5)	173 (28.3)	87 (23.8)	122 (20.2)	38 (17.0)	5 (3.0)	165 (18.7)	50 (10.8)	511 (39.5)	158 (25.9)	183 (34.2)	17 (11.8)	272 (27.3)	235 (35.1)	98 (25.3)	230 (11.6)	164 (36.3)	274 (9.8)	103 (26.4)	40 (18.6)
Section B	194 (22.9)	148 (11.6)	23 (12.8)	37 (9.7)	23 (12.6)	24 (7.8)	52 (8.5)	46 (12.6)	73 (12.1)	33 (14.8)	37 (22.3)	118 (13.4)	34 (7.3)	232 (17.9)	71 (11.6)	82 (15.3)	19 (13.2)	140 (14.1)	99 (14.8)	30 (7.8)	284 (14.3)	81 (17.9)	154 (5.5)	54 (13.8)	43 (20.0)
Section C	198 (23.4)	119 (9.3)	16 (8.9)	39 (10.2)	20 (10.9)	19 (6.2)	56 (9.2)	41 (11.2)	51 (8.5)	20 (9.0)	32 (19.3)	109 (12.4)	89 (19.2)	156 (12.1)	84 (13.7)	70 (13.1)	22 (15.3)	148 (14.9)	93 (13.9)	36 (9.3)	361 (18.2)	54 (11.9)	291 (10.4)	66 (16.9)	23 (10.7)
Section D	147 (17.4)	102 (8.0)	16 (8.9)	59 (15.4)	25 (13.7)	45 (14.6)	49 (8.0)	39 (10.7)	169 (28.0)	35 (15.7)	41 (24.7)	121 (13.7)	113 (24.4)	136 (10.5)	82 (13.4)	69 (12.9)	17 (11.8)	86 (8.6)	60 (9.0)	73 (18.9)	291 (14.6)	44 (9.7)	397 (14.2)	54 (13.8)	39 (18.1)
Section E (bottom)	154 (18.2)	621 (48.6)	92 (51.5)	212 (55.4)	77 (42.1)	197 (64.0)	282 (46.1)	153 (41.8)	188 (31.2)	97 (43.5)	51 (30.7)	368 (41.8)	178 (38.4)	259 (20.0)	216 (35.4)	131 (24.5)	69 (47.9)	350 (35.1)	183 (27.3)	150 (38.8)	822 (41.3)	109 (24.1)	1688 (60.2)	113 (29.0)	70 (32.6)
Number of larvae	846	1278	179	383	183	308	612	366	603	223	166	881	464	1294	611	535	144	996	670	387	1988	452	2804	390	215

Figure 26 Mean distributions of mature A. aspersa larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 26.

Table 89 Significances of differences in distributions (*G*-test) of mature *A. aspersa* larvae with a variety of hydrostatic pressures in the absence of light

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2		-																						
	3	*	*	-																					
0.5	1			*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1		*	*		*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*		*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*		*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*		*	*	*	*	*	*	*		*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*		*		*	*		*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05;

Table 90 Significances of differences in mean percentages (*t*-test) of mature *A. aspersa* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of hydrostatic pressures in the absence of light

(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

Applied hydrostatic pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	*	*	ns	ns	ns
0.5	ns	-	ns	ns	*	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	*	ns	ns	*	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Analysis of variance indicated that there were significant differences in the mean percentages of *A. aspersa* larvae found in the top ($F_{15, 32}$ = 6.30169, $p<0.001$) and bottom ($F_{15, 32}$ = 7.605423, $p<0.001$) sections of the vertical chamber (Table 91). Separate analysis of the data for young and mature larvae revealed significant differences in the mean percentages of young larvae found in the top ($F_{7, 16}$ = 13.78516, $p<0.001$) and the bottom ($F_{7, 16}$ = 29.79902, $p<0.001$) sections, but the mean percentages of mature larvae were not significantly different in either the top ($F_{7, 16}$ = 1.542083) or the bottom ($F_{7, 16}$ = 1.49635) sections (Table 91).

Table 91 ANOVA summary table

Sample	Source of variation	SS	df	MS	F
Young & mature *A. aspersa* (Top section of chamber)	Among groups	2429.551	15	161.970	6.30169
	Within groups	822.4844	32	25.70264	
	Total	3252.035	47		
Young & mature *A. aspersa* (Bottom section of chamber)	Among groups	2116.98	15	141.1321	7.605423
	Within groups	593.8164	32	18.55676	
	Total	2710.797	47		
Young *A. aspersa* (Top section of chamber)	Among groups	400.7367	7	57.24810	13.78516
	Within groups	66.44607	16	4.152879	
	Total	467.1828	23		
Young *A. aspersa* (Bottom section of chamber)	Among groups	605.0896	7	86.44137	29.79902
	Within groups	46.41300	16	2.900813	
	Total	651.5026	23		
Mature *A. aspersa* (Top section of chamber)	Among groups	510.0649	7	72.86641	1.542083
	Within groups	756.0311	16	47.25194	
	Total	1266.096	23		
Mature *A. aspersa* (Bottom section of chamber)	Among groups	385.6819	7	55.09741	1.49635
	Within groups	589.1393	16	36.82121	
	Total	974.8212	23		

A priori analysis of variance to compare the mean percentages of young and mature larvae in the top and bottom sections of the vertical chamber indicated a significant difference between the mean percentages of young and mature larvae found in the top section ($F_{1, 32}$ = 59.08924) of the chamber, and between the mean percentages of young and mature larvae found in the bottom section ($F_{1, 32}$ = 53.69254) of the chamber.

11.3.5 Young *Styela clava* larvae

The distributions of young *S. clava* larvae in the vertical behaviour chamber under applied hydrostatic pressure ranging from 0 to 3.5 m head of water, and in the absence of light, are presented in Table 92. Despite the large number of individual experiments with significantly different ($p<0.05$, *G*-test) distributions (Table 93) the mean larval distributions appear very similar, with over 70% of young larvae being found in the top section of the chamber irrespective of the applied hydrostatic pressure (Figure 27). This indicates that the negative geotactic response of the larvae is not affected by change in hydrostatic pressure over the pressure range studied. The mean percentages of young larvae in the bottom section of the chamber did not vary significantly except between the extremes of hydrostatic pressure (Table 94).

11.3.6 Mature *Styela clava* larvae

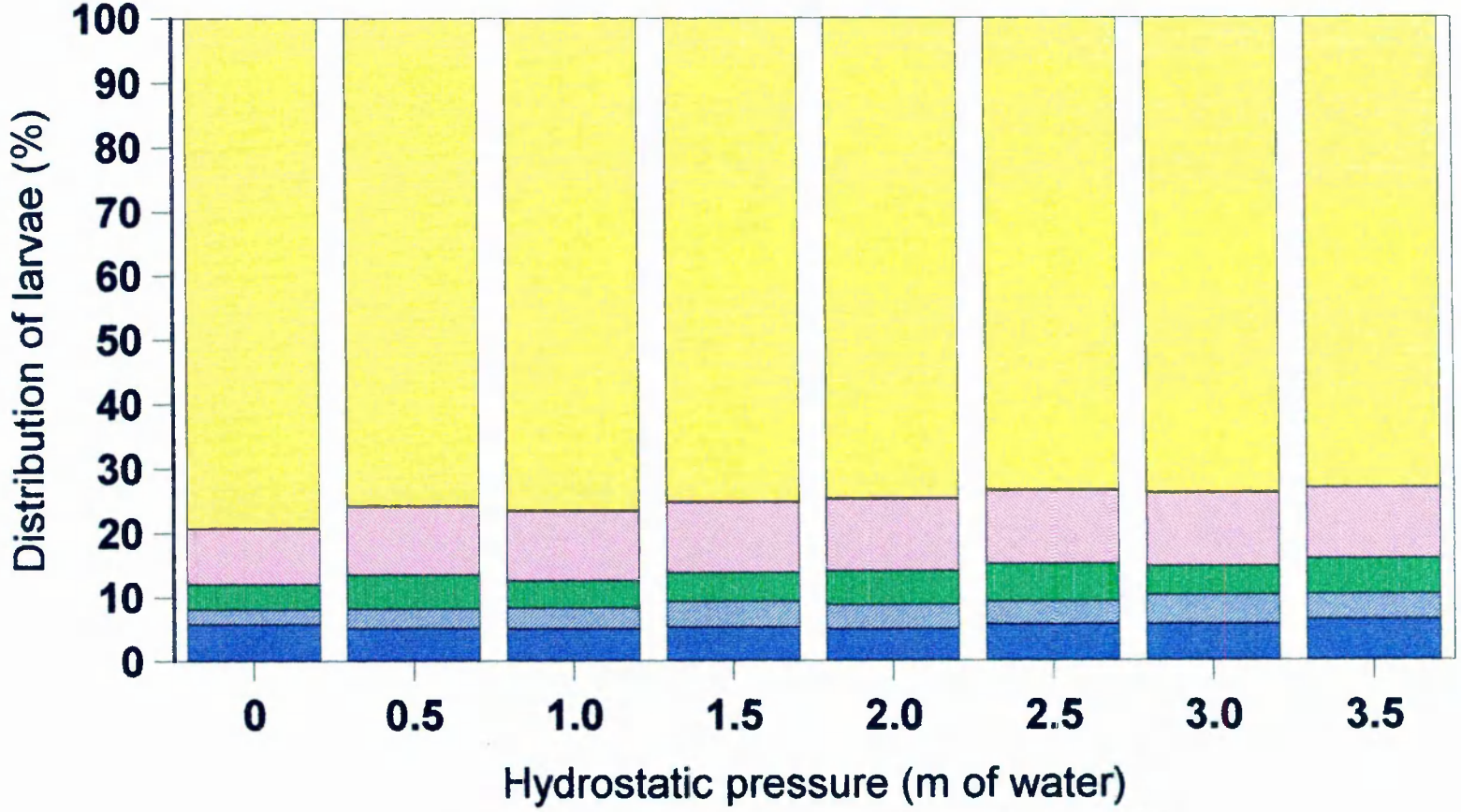
The distributions of mature *S. clava* larvae in the vertical behaviour chamber under applied hydrostatic pressure ranging from 0 to 3.5 m head of water, and in the absence of light, are presented in Table 95; more distributions were significantly different ($p<0.05$, *G*-test) than for young larvae (Table 96). Smaller proportions of mature larvae rose to the top section than for young larvae, suggesting a reduced negative geotactic response in the population.

Mean larval distributions show a slight decline in the proportion of mature larvae in the top section of the chamber between 0 m and 1.5 m head of water (Figure 28), but the mean percentages of larvae in either the top or bottom sections of the chamber were not significantly different ($p>0.05$, *t*-test) irrespective of the applied pressures (Table 97).

Table 92 **Distribution (and %) of young *S. clava* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																							
		0 (Table 38)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	489 (16.1)	900 (79.1)	7049 (79.5)	680 (78.7)	1621 (76.9)	637 (70.2)	4245 (76.3)	974 (76.8)	414 (77.2)	313 (76.2)	536 (74.8)	1041 (75.2)	450 (75.8)	1567 (74.4)	1255 (75.0)	933 (74.8)	1038 (73.6)	388 (72.5)	354 (74.7)	546 (73.3)	1677 (74.1)	340 (73.8)	1778 (73.2)	353 (72.5)	424 (73.6)
Section B	708 (23.4)	93 (8.2)	760 (8.6)	88 (10.2)	207 (9.8)	105 (11.6)	607 (10.9)	141 (11.1)	55 (10.3)	45 (10.9)	82 (11.4)	152 (11.0)	62 (10.4)	238 (11.3)	184 (11.0)	145 (11.6)	158 (11.2)	63 (11.8)	55 (11.6)	82 (11.0)	262 (11.6)	52 (11.3)	260 (10.7)	58 (11.9)	65 (11.3)
Section C	679 (22.4)	48 (4.2)	345 (3.9)	40 (4.6)	107 (5.1)	85 (9.4)	273 (4.9)	51 (4.0)	20 (3.7)	21 (5.1)	37 (5.2)	50 (3.6)	35 (5.9)	129 (6.1)	75 (4.5)	64 (5.1)	82 (5.8)	40 (7.5)	20 (4.2)	49 (6.6)	86 (3.8)	21 (4.6)	143 (5.9)	26 (5.3)	25 (4.3)
Section D	566 (18.7)	22 (1.9)	196 (2.2)	19 (2.2)	65 (3.1)	37 (4.1)	145 (2.6)	37 (2.9)	21 (3.9)	12 (2.9)	24 (3.3)	66 (4.8)	18 (3.0)	63 (3.0)	74 (4.5)	49 (3.9)	44 (3.1)	19 (3.6)	22 (4.6)	35 (4.7)	97 (4.3)	25 (5.4)	92 (3.8)	19 (3.9)	26 (4.5)
Section E (bottom)	588 (19.4)	75 (6.6)	522 (5.9)	37 (4.3)	108 (5.1)	44 (4.8)	295 (5.3)	66 (5.2)	26 (4.9)	20 (4.9)	38 (5.3)	75 (5.4)	29 (4.9)	110 (5.2)	85 (5.1)	57 (4.6)	89 (6.3)	25 (4.7)	25 (5.3)	33 (4.4)	140 (6.2)	23 (5.0)	155 (6.4)	31 (6.4)	36 (6.3)
Number of larvae	3030	1138	8872	864	2108	908	5565	1269	536	411	717	1384	594	2107	1674	1248	1411	535	476	745	2262	461	2428	487	576

Figure 27 Mean distributions of young S. clava larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 27.

Table 93 **Significances of differences in distributions (*G*-test) of young *S. clava* larvae with a variety of applied hydrostatic pressures in the absence of light**

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*		-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*			*	*	-																	
	2	*				*	*		-																
	3					*	*			-															
1.5	1	*	*			*					-														
	2	*	*	*	*	*	*	*		*	*	-													
	3	*	*			*	*			*		*	-												
2.0	1	*	*	*	*	*		*	*			*		-											
	2	*	*	*	*	*	*	*		*			*	*	-										
	3	*	*	*		*	*					*				-									
2.5	1	*	*	*	*	*		*	*			*			*	*	-								
	2	*	*	*			*	*	*			*			*			-							
	3	*	*	*		*												*	-						
3.0	1	*	*	*	*		*	*	*	*		*					*		*	-					
	2	*	*	*	*	*	*	*	*	*	*		*	*		*	*	*		*	-					
	3	*	*	*		*	*											*				-				
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
	2	*	*	*		*											*	*	*					-		
	3	*	*	*		*											*	*	*						-	

□= not significant (p>0.05); * = p<0.05;

Table 94 **Significances of differences in mean percentages (*t*-test) of young *S. clava* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of applied hydrostatic pressures in the absence of light**

(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

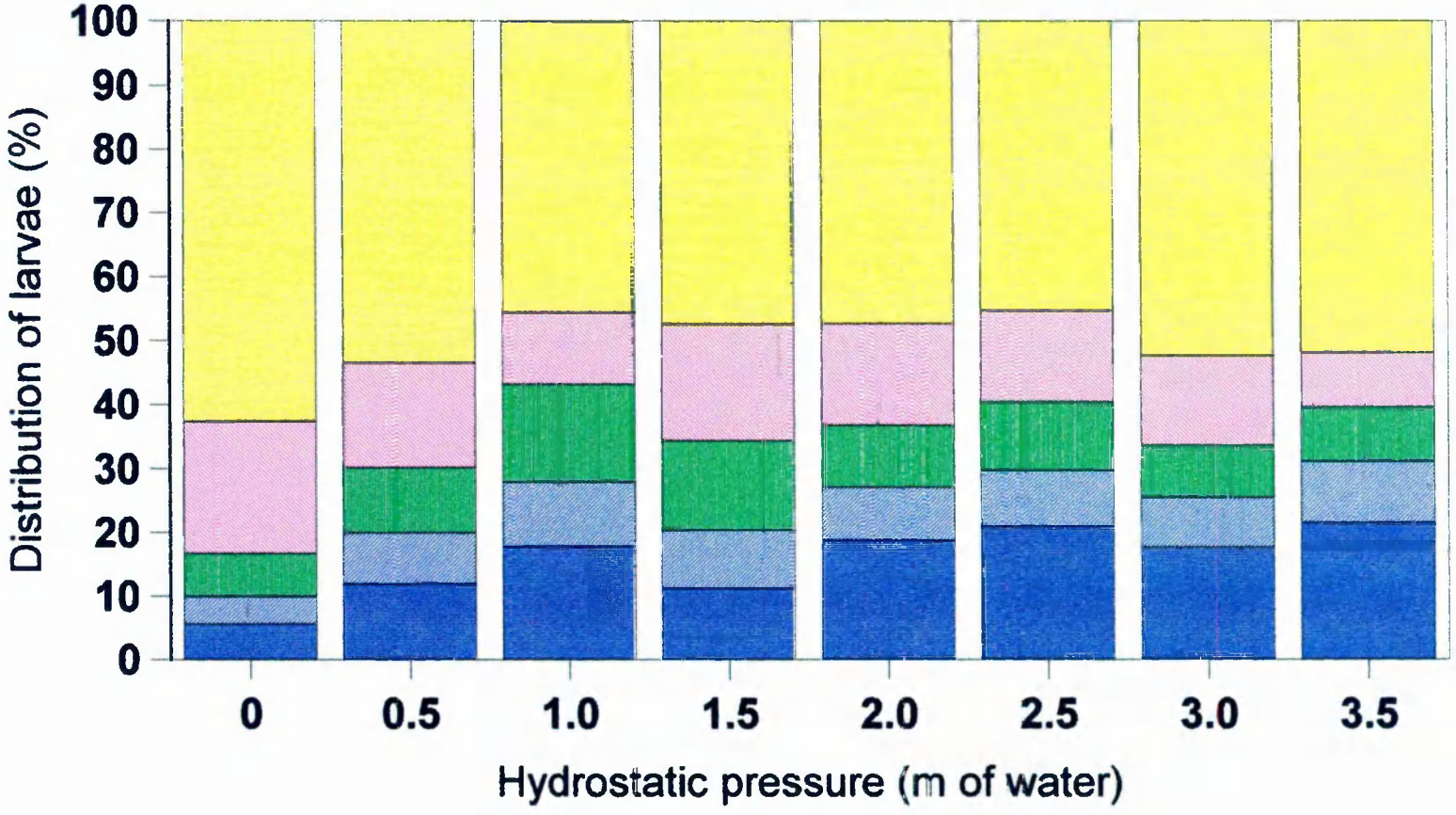
Applied hydrostatic pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	*
1.0	**	ns	-	ns	ns	ns	ns	**
1.5	***	ns	*	-	ns	ns	ns	*
2.0	ns	ns	*	ns	-	ns	ns	*
2.5	**	ns	*	ns	ns	-	ns	ns
3.0	***	ns	**	*	*	ns	-	ns
3.5	***	ns	**	**	*	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 95 **Distribution (and %) of mature *S. clava* larvae in the vertical behaviour chamber under a variety of hydrostatic pressure conditions in the absence of light**

		Hydrostatic pressure (m head of water)																							
		0 (Table 39)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Control	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Section A (top)	489 (16.1)	154 (50.3)	3324 (64.9)	343 (51.8)	904 (49.5)	373 (50.9)	535 (64.7)	235 (48.3)	3607 (40.5)	3374 (52.5)	167 (52.0)	343 (45.9)	2277 (47.4)	42 (35.3)	430 (50.1)	1416 (47.1)	364 (54.2)	109 (39.8)	1047 (43.5)	597 (51.3)	244 (59.7)	259 (49.2)	289 (57.0)	159 (55.2)	113 (40.2)
Section B	708 (23.4)	51 (16.7)	1101 (21.5)	102 (15.4)	323 (17.7)	129 (17.6)	105 (12.7)	43 (8.8)	692 (7.8)	1024 (15.9)	62 (19.3)	92 (12.3)	911 (19.0)	26 (21.8)	149 (17.4)	460 (15.3)	121 (18.0)	22 (8.0)	337 (14.0)	172 (14.8)	34 (8.3)	88 (16.7)	29 (5.7)	42 (14.6)	20 (7.1)
Section C	679 (22.4)	30 (9.8)	313 (6.1)	71 (10.7)	206 (11.3)	84 (11.5)	55 (6.7)	56 (11.5)	1492 (16.8)	874 (13.6)	31 (9.7)	68 (9.1)	728 (15.2)	24 (20.2)	87 (10.1)	272 (9.1)	55 (8.2)	17 (6.2)	283 (11.8)	96 (8.3)	32 (7.8)	43 (8.2)	45 (8.9)	26 (9.0)	19 (6.8)
Section D	566 (18.7)	30 (9.8)	180 (3.5)	53 (8.0)	159 (8.7)	57 (7.8)	56 (6.8)	44 (9.0)	1044 (11.7)	512 (8.0)	19 (5.9)	8.3 (11.1)	426 (8.9)	10 (8.4)	69 (8.0)	254 (8.4)	50 (7.5)	27 (9.9)	219 (9.1)	113 (9.7)	19 (4.6)	32 (6.1)	53 (10.5)	20 (6.9)	30 (10.7)
Section E (bottom)	588 (19.4)	41 (13.4)	207 (4.0)	93 (14.0)	236 (12.9)	90 (12.3)	76 (9.2)	109 (22.4)	2064 (23.2)	648 (10.1)	42 (13.1)	161 (21.6)	462 (9.6)	17 (14.3)	123 (14.3)	604 (20.1)	81 (12.1)	99 (36.1)	519 (21.6)	185 (15.9)	80 (19.6)	104 (19.8)	91 (17.9)	41 (14.2)	99 (35.2)
Number of larvae	3030	306	5125	662	1828	733	827	487	8899	6432	321	747	4804	119	858	3006	671	274	2405	1163	409	526	507	288	281

Figure 28 Mean distributions of mature S. clava larvae with hydrostatic pressure in the absence of light



See Figure 10 (page 88) for convention used in Figure 28.

Table 96 **Significances of differences in distributions (*G*-test) of mature *S. clava* larvae with a variety of applied hydrostatic pressures in the absence of light**

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3		*	-																					
0.5	1		*	*	-																				
	2		*			-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1		*				*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*		-											
	2		*				*	*	*	*		*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1		*	*	*		*	*	*	*		*	*	*		*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	-		
	2		*				*	*	*	*	*	*	*	*		*		*	*		*		*	-		
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	-	

□= not significant (p>0.05); * = p<0.05;

Table 97 **Significances of differences in mean percentages (*t*-test) of mature *S. clava* larvae in top (A) and bottom (E) sections of the behaviour chamber with a variety of applied hydrostatic pressures in the absence of light**

(Left-hand side of table (clear) = section A; right-hand side of table (shaded) = section E)

Applied hydrostatic pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	*	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Analysis of variance indicated that there were significant differences in the mean percentages of *S. clava* larvae found in the top ($F_{15, 32}$ = 21.09657, p<0.001) and bottom ($F_{15, 32}$ = 7.24983, p<0.001) sections of the vertical chamber (Table 98). Separate analysis of the data for young and mature larvae revealed significant differences in the mean percentages of young larvae found in the top section ($F_{7, 16}$ = 6.25446, p<0.001) but not in the bottom section ($F_{7, 16}$ = 1.53032), and no significant differences in the mean percentages of mature larvae found in either the top ($F_{7, 16}$ = 1.09002) or the bottom ($F_{7, 16}$ = 1.33930) sections (Table 98).

Table 98 ANOVA summary table

Sample	Source of variation	SS	df	MS	F
Young & mature *S. clava* (Top section of chamber)	Among groups	2886.203	15	192.4135	21.09657
	Within groups	291.8594	32	9.120605	
	Total	3178.063	47		
Young & mature *S. clava* (Bottom section of chamber)	Among groups	1600.434	15	106.6956	7.24983
	Within groups	470.9434	32	14.71698	
	Total	2071.377	47		
Young *S. clava* (Top section of chamber)	Among groups	37.13281	7	5.304688	6.25446
	Within groups	13.57031	16	0.848145	
	Total	50.70313	23		
Young *S. clava* (Bottom section of chamber)	Among groups	6.88020	7	0.98289	1.53032
	Within groups	10.27640	16	0.64228	
	Total	17.15660	23		
Mature *S. clava* (Top section of chamber)	Among groups	132.7148	7	18.95926	1.09002
	Within groups	278.2969	16	17.39355	
	Total	411.0117	23		
Mature *S. clava* (Bottom section of chamber)	Among groups	269.9248	7	38.56068	1.33930
	Within groups	460.6663	16	28.79105	
	Total	730.5911	23		

A priori analysis of variance to compare the mean percentages of young and mature larvae in the top and bottom sections of the chamber over the full range of hydrostatic pressures, indicated that there were significant differences between the mean percentages of young and mature larvae found in the top section ($F_{1, 32}$ = 297.8283, p<0.001) and in the bottom section ($F_{1, 32}$ = 89.939, p<0.001) of the vertical chamber.

11.4 Discussion

The proportions of the populations of young and mature *C. intestinalis* larvae that exhibited active negative geotaxis declined with increasing applied hydrostatic pressure to a minimum at about 2 m head of water. At hydrostatic pressures greater than 2.5 m, the proportions of the larval populations exhibiting negative geotaxis increased with pressure to at least 3.5 m head of water. These changes in the distribution of *C. intestinalis* larvae with hydrostatic pressure suggest that, in darkness, a circulation cell could be formed in the water column, with larvae subjected to a hydrostatic pressure of at least 4.5 m rising through the water column to around 2 m depth whereupon many begin to sink again. How could this circulation be controlled?

Any mechanisms proposed to explain the operation of such a circulation cell must involve a response threshold being exceeded in an increasing proportion of the population as pressure increases; then as the larvae rise, the response threshold would be crossed again and the response would cease. Since gravity is a constant force, the change in response cannot involve a geotactic threshold; there must be a barokinetic response involved, and it would appear that *C. intestinalis* larvae exhibit high barokinesis. As larvae sink a barokinetic threshold is exceeded and motion is initiated, the direction being governed by the larval geotactic response which, being negative (Chapter 8), results in a net upward movement of larvae. As the larvae rise, the barokinetic response threshold is crossed again, active motion ceases and the larvae sink under the effect of negative buoyancy (Chapter 7) until the increased hydrostatic pressure triggers a new cycle of movement. Hydrostatic pressures of less than 1 m head of water appear to have little effect on the distribution of *C. intestinalis* larvae in darkness so those larvae that reach the surface layers will, in the absence of any

other clues, tend to stay in the vicinity of the surface. However, it is unlikely that the surface layers would be in complete darkness and, depending upon the light flux encountered, some larvae would be induced to sink (Chapter 10) and possibly rejoin the circulation cell.

The negative geotactic response of young *A. aspersa* larvae was only slightly affected by increase of hydrostatic pressure between 0 m and 2.5 m head of water; the maximum response occurred at 2 m hydrostatic pressure. Mature *A. aspersa* larvae showed a more distinct increase in negative geotaxis with increasing hydrostatic pressure between 0 m and 2.5 m head of water. As with the young larvae, the maximum negative geotactic response was observed at 2.0 m head of water. Both young and old larvae tended to sink at hydrostatic pressures greater than 2.5 m. This sinking response could be the result of a change in the direction of the geotactic response of a proportion of the population, a change in the balance of the proportions of positively geotactic and negatively geotactic larvae in a mixed population, a change from high barokinesis to low barokinesis or the cessation of swimming activity. A change in the direction or balance of geotaxis of part of the population would produce active migration, whereas a change in barokinesis or cessation of swimming would produce passive migration (resulting from the negative buoyancy of the inactive larvae). The variation in the proportion of mature larvae in the bottom section of the chamber (Figure 26) is similar in trend, but smaller in magnitude, to that of anaesthetised larvae (Figure 14, Chapter 7), suggesting that passive migration is occurring.

Over 70% of young *S. clava* larvae were found in the top section of the chamber irrespective of the applied hydrostatic pressure, indicating that the negative geotactic response of these larvae is not affected by this magnitude of pressure increase. Smaller proportions of the population of mature larvae than of young larvae were found in the top

section of the chamber over the range of applied hydrostatic pressures, suggesting that the negative geotactic response declines with age. Mean distributions showed a slight decline in the proportion of mature larvae in the top section of the chamber, and an increase in the proportion in the bottom section, between 0 m and 2.0 m head of water, but there was no clear relationship between larval distribution and applied hydrostatic pressure.

The larvae of these three ascidian species appear to react very differently to changes in applied hydrostatic pressure in the absence of light. The negative geotactic response of *S. clava* larvae will cause them to concentrate near the surface. The minimum negative geotactic response for *C. intestinalis* larvae occurred at hydrostatic pressures of around 2 m head of water, the hydrostatic pressure that produced the maximum negative geotactic response for *A. aspersa* larvae. This suggests that, in darkness, larvae of these two species would tend to avoid each other by concentrating at different depths in the water column.

Whilst behavioural explanations based on geotaxis and barokinesis can adequately account for the observed larval distributions, it should be noted that the pressure changes employed were discrete and static, i.e. the experiments determined the distribution of larvae suddenly exposed to a positive hydrostatic pressure and held at that pressure, and at constant temperature, for an hour. Under natural conditions, the pressure range is continuous and changes are dynamic, i.e. pressure changes as the larvae rise or sink (as does temperature, but less predictably), so feedback control of depth by larvae may be possible. Nevertheless, the distributions of *C. intestinalis* and *A. aspersa* larvae, but not *S. clava* larvae, change in a metre column of water when the applied pressure is increased beyond 2 m head of water. The change in *C. intestinalis* distribution can only be achieved by active movement, but it is not yet clear whether the downward movement of *A. aspersa* larvae is active or passive.

CHAPTER 12 LARVAL RESPONSE TO GRAVITY, LIGHT AND PRESSURE IN COMBINATION

12.1 Introduction

When light was applied in combination with gravity to larval populations of the three ascidian species, *A. aspersa* larvae exhibited positive phototaxis, *S. clava* larvae exhibited negative phototaxis and *C. intestinalis* larvae exhibited ambivalent phototaxis (Chapter 10). In this chapter hydrostatic pressure will be incorporated with these two cues. A comparison of the two sets of responses should permit an assessment of the contribution of hydrostatic pressure to the observed distribution when the three cues operate in unison, as they do in the natural environment.

When hydrostatic pressure and gravity were applied to populations of larvae, *S. clava* larvae showed no variation in negative geotactic response with increasing pressure, but the initial negative geotactic response of *A. aspersa* larvae was attenuated, and that of *C. intestinalis* was enhanced (Chapter 11). Light is added to these two cues in the experiments described in this chapter; therefore, a comparison of these two sets of responses should permit an assessment of the contribution of light to the observed distribution when the three cues operate in unison.

The responses to the three cues in unison will be determined by repeating the gravity plus light experiments (Chapter 10) over a range of static applied hydrostatic pressures from 0 m to 3.5 m head of water.

12.2 Methods

The vertical behaviour chamber was deployed and operated as in section 10.2.1. The valve connecting the variable head tube and the chamber was closed and the tube filled with filtered (10 μm) sea water. The chamber was filled to overflowing with a suspension of the ascidian larvae (approximately 350 ml) and the window screwed onto the top of the tube in such a way that the amount of trapped air was kept to a minimum. The chamber was inverted five times and fixed vertically. The height of variable head tube was adjusted to produce the required hydrostatic head and the valve was opened. The chamber was left at constant temperature for one hour, with the light level monitored every few minutes; the valves were then closed and each segment of water was decanted in turn, with rinsing. The samples were stained and preserved for later examination (section 5.2).

The balanced experimental design, with crossed factors, permitted two-way analysis of variance (Model 1) of the percentages of larvae (with arcsine transformation) in a specific section of the vertical behaviour chamber for the 5 x 8 factorial experiment.

12.3 Results

12.3.1 *Ciona intestinalis* larvae

The distributions of *C. intestinalis* larvae in the vertical behaviour chamber with a light flux of 250 lux and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 99. The significances of the differences (*G*-test) of the distributions are presented in Table 100.

Table 99 **Distribution (and %) of mature *C. intestinalis* larvae in the vertical behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures**

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 64)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177	1611	1023	1028	350	1173	523	572	520	1075	472	348	396	768	368	632	135	427	699	295	622	416	658	149	108
	(20.8)	(39.7)	(36.8)	(21.6)	(41.2)	(24.4)	(30.5)	(24.3)	(27.8)	(13.9)	(19.5)	(11.7)	(18.9)	(24.2)	(8.5)	(5.9)	(4.8)	(14.9)	(6.1)	(7.1)	(11.5)	(9.8)	(20.0)	(12.0)	(9.0)
Section B	150	536	391	902	149	1012	256	525	249	1114	305	397	226	491	338	946	188	256	1323	508	649	539	377	182	224
	(17.6)	(13.2)	(14.1)	(18.9)	(17.6)	(21.1)	(15.0)	(22.3)	(13.3)	(14.4)	(12.6)	(13.4)	(10.8)	(15.5)	(7.8)	(8.8)	(6.7)	(8.9)	(11.5)	(12.2)	(12.0)	(12.7)	(11.4)	(14.7)	(18.6)
Section C	148	750	308	829	71	962	172	254	275	1113	256	371	262	213	400	1252	256	306	1582	597	607	437	341	141	165
	(17.4)	(18.5)	(11.1)	(17.4)	(8.4)	(20.0)	(10.0)	(10.8)	(14.7)	(14.4)	(10.6)	(12.5)	(12.5)	(6.7)	(9.2)	(11.7)	(9.1)	(10.7)	(13.7)	(14.4)	(11.3)	(10.3)	(10.3)	(11.4)	(13.7)
Section D	196	429	303	728	90	533	217	218	211	1247	367	356	297	186	401	685	254	287	1568	580	544	443	489	153	182
	(23.0)	(10.6)	(10.9)	(15.3)	(10.6)	(11.1)	(12.7)	(9.3)	(11.3)	(41.0)	(15.2)	(12.0)	(14.2)	(5.9)	(9.2)	(6.4)	(9.1)	(10.0)	(13.6)	(14.0)	(10.1)	(10.4)	(14.8)	(12.3)	(15.1)
Section E (bottom)	182	729	755	1282	189	1126	544	782	618	3166	1022	1499	910	1512	2831	7218	1971	1586	6367	2174	2971	2412	1433	616	526
	(21.3)	(18.0)	(27.2)	(26.9)	(22.3)	(23.4)	(31.8)	(33.3)	(33.0)	(41.0)	(42.2)	(50.5)	(43.5)	(47.7)	(65.3)	(67.3)	(70.3)	(55.4)	(55.2)	(52.3)	(55.1)	(56.8)	(43.5)	(49.6)	(43.7)
Number of larvae	853	4055	2780	4769	849	4806	1712	2351	1873	7715	2422	2971	2091	3170	4338	10733	2804	2862	11539	4154	5393	4247	3298	1241	1205

Table 100 Significances of differences in distributions (*G*-test) of *C. intestinalis* larvae with 250 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

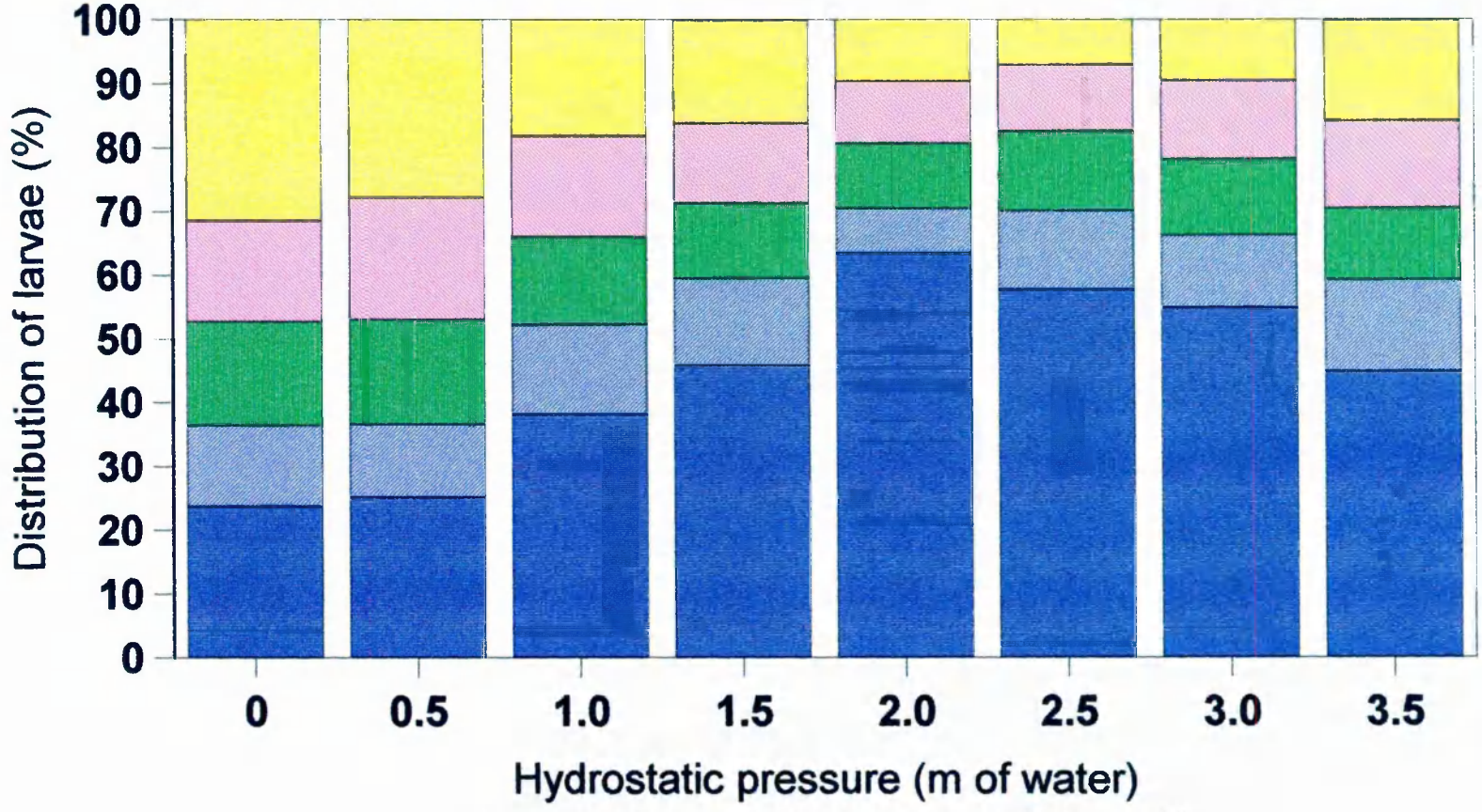
Table 101 Significances of differences in mean percentages (*t*-test) of *C. intestinalis* larvae in section A (top) and section E (bottom) of the behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	**	*	**	**	*
0.5	ns	-	ns	**	*	**	**	*
1.0	ns	ns	-	ns	*	*	**	*
1.5	ns	ns	ns	-	ns	ns	*	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	*	*	ns	ns	ns	-	ns	ns
3.0	*	*	ns	ns	ns	ns	-	*
3.5	*	*	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Figure 29 Mean distributions of mature C. intestinalis larvae exposed to 250 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 29.

The mean distributions of *C. intestinalis* larvae in the vertical chamber with a light flux of 250 lux intensity applied over the range of pressures (Figure 29) were similar to those observed in the absence of light (Figures 23 and 24), with the proportion of larvae in the bottom section of the chamber increasing with applied hydrostatic pressure to a maximum at 2 m head of water then declining. However the decline in the proportion of larvae in the bottom section of the chamber, and the commensurate increase in the proportion of larvae in the top section, at pressures greater than 2 m head of water was not as great with a light flux of 250 lux intensity as it was in the absence of light.

With a light flux of 250 lux intensity applied from above, the majority of significant differences in the mean percentages of *C. intestinalis* larvae in the end sections of the vertical chamber occurred in the bottom section (Table 101), suggesting that the middle sections buffer any changes in the larval distribution.

The distributions of *C. intestinalis* larvae in the vertical behaviour chamber resulting from exposure to a light flux of 500 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 102. Despite the vast majority of larval distributions being significantly different ($p<0.05$, *G*-test) from each other (Table 103), the mean distributions of *C. intestinalis* larvae in the vertical chamber are all very similar, with approximately 10% of larvae in the top section of the chamber and 50% in the bottom section (Figure 30). The similarity in the distributions is also as indicated by the lack of significant differences in the mean percentages of larvae in the end sections of the vertical chamber (Table 104).

Table 102 Distribution (and %) of mature *C. intestinalis* larvae in the vertical behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																								
	Control	0 (Table 65)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	41 (13.1)	197 (12.3)	681 (9.4)	379 (10.3)	199 (10.8)	358 (12.6)	127 (5.4)	77 (12.0)	1113 (21.8)	180 (14.6)	132 (13.8)	448 (6.3)	259 (13.6)	589 (6.7)	281 (11.6)	464 (8.0)	1154 (23.6)	505 (8.3)	71 (6.1)	293 (15.6)	186 (10.5)	882 (10.9)	765 (18.2)	216 (6.0)
Section B	150 (17.6)	46 (14.7)	55 (3.4)	898 (12.4)	366 (9.9)	148 (8.0)	257 (9.1)	305 (12.9)	105 (16.3)	719 (14.1)	112 (9.1)	152 (15.9)	405 (5.8)	269 (14.1)	925 (10.5)	286 (11.8)	1127 (19.4)	626 (12.8)	659 (10.9)	131 (11.1)	300 (15.9)	218 (12.3)	892 (11.1)	484 (11.5)	472 (13.0)
Section C	148 (17.4)	37 (11.9)	91 (5.7)	848 (11.7)	425 (11.5)	208 (11.2)	344 (12.1)	392 (16.6)	103 (16.0)	515 (10.1)	131 (10.6)	184 (19.2)	735 (10.4)	262 (13.8)	1214 (13.8)	302 (12.5)	1251 (21.6)	692 (14.2)	737 (12.2)	156 (13.4)	228 (12.1)	231 (13.0)	976 (12.1)	406 (9.6)	463 (12.8)
Section D	196 (23.0)	28 (9.0)	186 (11.6)	831 (11.5)	491 (13.3)	233 (12.6)	456 (16.1)	504 (21.4)	103 (16.0)	587 (11.5)	197 (16.0)	180 (18.8)	1457 (20.7)	172 (9.0)	1457 (16.6)	353 (14.6)	1098 (18.9)	493 (10.1)	1001 (16.5)	164 (14.1)	285 (15.1)	250 (14.1)	1068 (13.3)	505 (12.0)	507 (14.0)
Section E (bottom)	182 (21.3)	160 (51.3)	1076 (67.0)	3996 (55.1)	2034 (55.0)	1063 (57.4)	1421 (50.1)	1029 (43.7)	256 (39.8)	2174 (42.6)	614 (49.8)	309 (32.3)	3992 (56.7)	942 (49.5)	4595 (52.3)	1202 (49.6)	1857 (32.0)	1921 (39.3)	3157 (52.1)	645 (55.3)	778 41.3()	892 (50.2)	4249 (52.7)	2054 (48.7)	1963 (54.2)
Number of larvae	853	312	1605	7254	3695	1851	2836	2357	644	5108	1234	957	7037	1904	8780	2424	5797	4886	6059	1167	1884	1777	8067	4214	3621

Table 103 Significances of differences in distributions (*G*-test) of *C. intestinalis* larvae with 500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*		*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1		*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

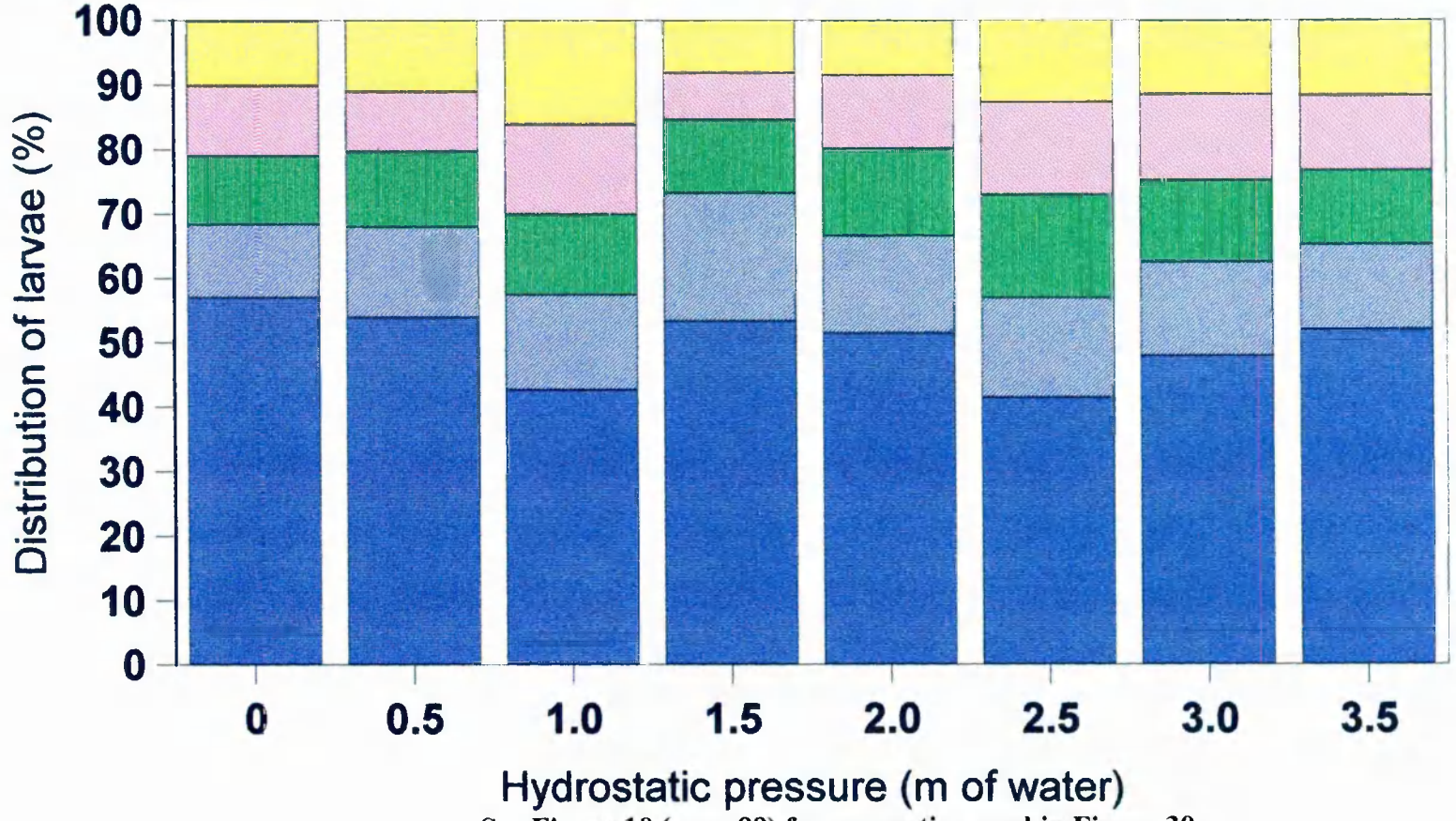
Table 104 Significances of differences in mean percentages (*t*-test) of *C. intestinalis* larvae in section A (top) and section E (bottom) of the behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	*	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	**	ns	ns	**
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Figure 30 Mean distributions of mature C. intestinalis larvae exposed to 500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 30.

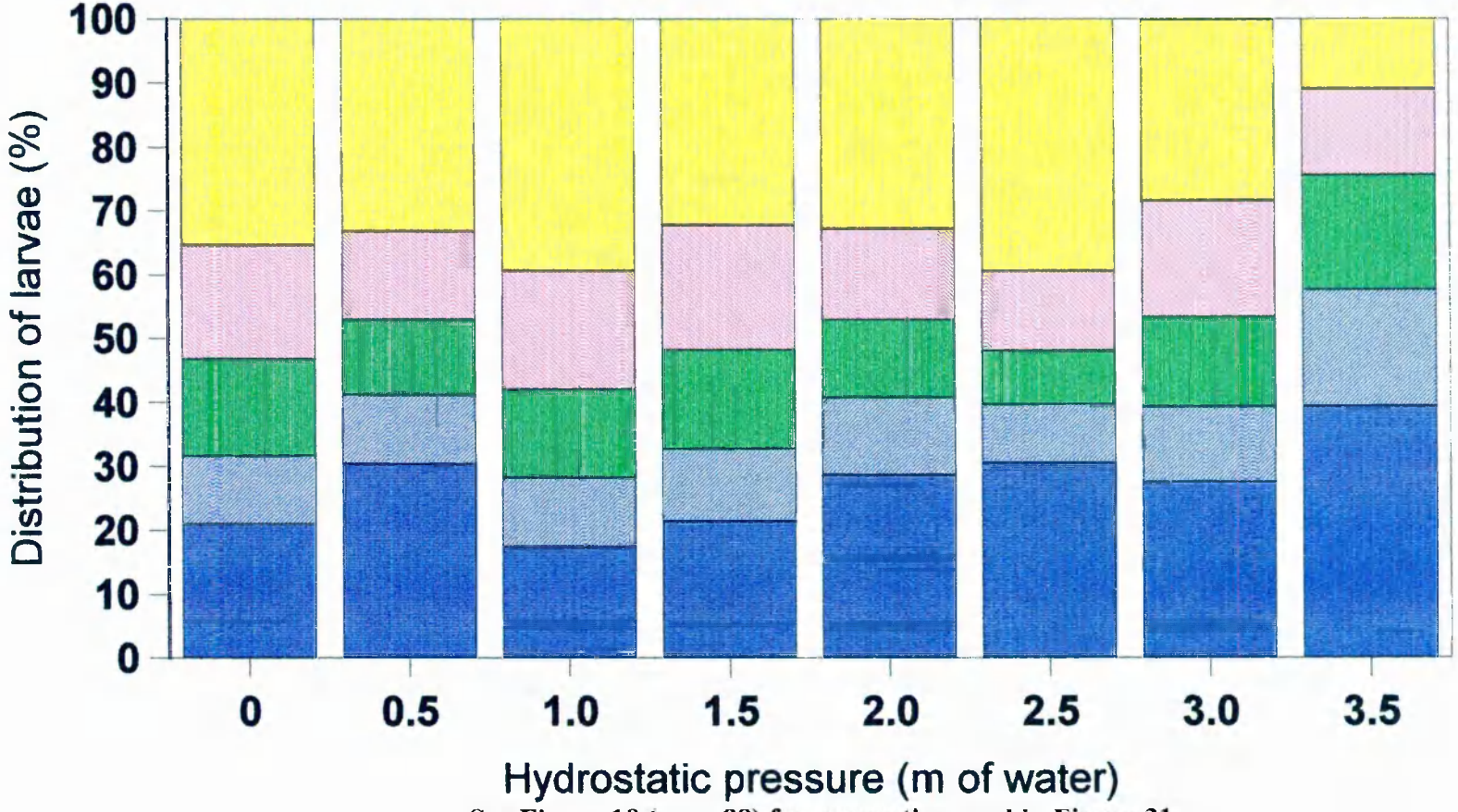
The distributions of *C. intestinalis* larvae in the vertical behaviour chamber with a light flux of 1000 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 105. The mean distributions of *C. intestinalis* larvae (Figure 31) show an increased proportion of larvae in the top section of the chamber, and a reduced proportion of larvae in the bottom section of the chamber, compared with the distributions observed with light fluxes of 250 and 500 lux intensity. The changes in larval distributions relative to those observed at lower light intensities becomes less at higher applied hydrostatic pressures, and at 3.5 m head of water the distributions at all three light intensities are similar. Although the larval distributions are generally significantly different ($p<0.05$, *G*-test) from each other (Table 106), the differences in the mean percentages of larvae in the top and bottom sections of the vertical behaviour chamber (Table 107) are generally not significant ($p>0.05$, *t*-test).

The distributions of *C. intestinalis* larvae in the vertical behaviour chamber with a light flux of 1500 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 108. The mean distributions of *C. intestinalis* larvae in the vertical chamber show an increased proportion of larvae in the top section of the chamber, and a reduced proportion of larvae in the bottom section of the chamber, compared with all other distributions (Figure 32). However, despite the majority of the distributions being significantly different ($p<0.05$, *G*-test) from each other (Table 109), only two of the differences in the mean percentages of larvae in the end sections of the chamber are significant ($p<0.05$, *t*-test); both of these significant differences larvae occurring in the top section of the behaviour chamber (Table 110).

Table 105 **Distribution (and %) of mature *C. intestinalis* larvae in the vertical behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures**

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 66)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	153 (18.1)	206 (39.9)	845 (32.0)	3429 (36.2)	216 (31.9)	669 (34.1)	191 (31.7)	1597 (38.3)	1045 (40.9)	581 (39.9)	746 (31.9)	2898 (32.4)	167 (32.5)	520 (20.8)	1717 (37.8)	272 (45.8)	162 (32.1)	924 (43.7)	519 (35.8)	534 (27.4)	4270 (28.4)	236 (34.7)	58 (23.0)	259 (8.6)	1021 (11.6)
Section B	194 (22.9)	70 (13.6)	314 (11.9)	1863 (19.6)	112 (16.5)	270 (13.7)	64 (10.6)	843 (20.2)	464 (18.2)	216 (14.8)	356 (15.2)	1874 (21.0)	68 (13.3)	391 (15.6)	607 (13.4)	86 (14.5)	83 (16.5)	204 (9.7)	221 (15.2)	469 (21.0)	2661 (17.7)	70 (10.3)	22 (8.7)	468 (15.6)	1122 (12.8)
Section C	198 (23.4)	49 (9.5)	364 (13.8)	1509 (15.9)	100 (14.8)	220 (11.2)	63 (10.5)	521 (12.5)	360 (14.1)	249 (17.1)	408 (17.4)	1352 (15.1)	66 (12.9)	331 (13.2)	547 (12.1)	52 (8.8)	60 (11.9)	152 (7.2)	131 (9.0)	272 (13.9)	2144 (14.3)	80 (11.8)	46 (18.3)	648 (21.6)	1477 (16.8)
Section D	147 (17.4)	50 (9.7)	302 (11.4)	983 (10.4)	66 (9.7)	200 (10.2)	85 (14.1)	455 (10.9)	265 (10.4)	163 (11.2)	359 (15.3)	901 (10.1)	66 (12.9)	264 (10.6)	616 (13.6)	39 (6.6)	56 (11.1)	149 (7.1)	165 (11.4)	161 (8.2)	1818 (12.1)	86 (12.6)	40 (15.9)	758 (25.3)	1393 (15.8)
Section E (bottom)	154 (18.2)	141 (27.3)	818 (30.9)	1699 (17.9)	183 (27.0)	605 (30.8)	199 (33.1)	755 (18.1)	418 (16.4)	246 (16.9)	471 (20.1)	1913 (21.4)	146 (28.5)	993 (39.7)	1051 (23.2)	145 (24.4)	143 (28.4)	684 (32.4)	415 (28.6)	516 (26.4)	4139 (27.5)	208 (30.6)	86 (34.1)	864 (28.8)	3783 (43.0)
Number of larvae	846	516	2643	9483	677	1964	602	4171	2552	1455	2340	8938	513	2499	4538	594	504	2113	1451	1952	15032	680	252	2997	8796

Figure 31 Mean distributions of mature C. intestinalis larvae exposed to 1000 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 31.

Table 106 **Significances of differences in distributions (*G*-test) of *C. intestinalis* larvae with 1000 lux light flux and a variety of applied hydrostatic pressures**

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*		*	*		*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*			*	*	*	*	*	*		*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*		*	*	*		*	*	*	*	*		*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 107 **Significances of differences in mean percentages (*t*-test) of *C. intestinalis* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

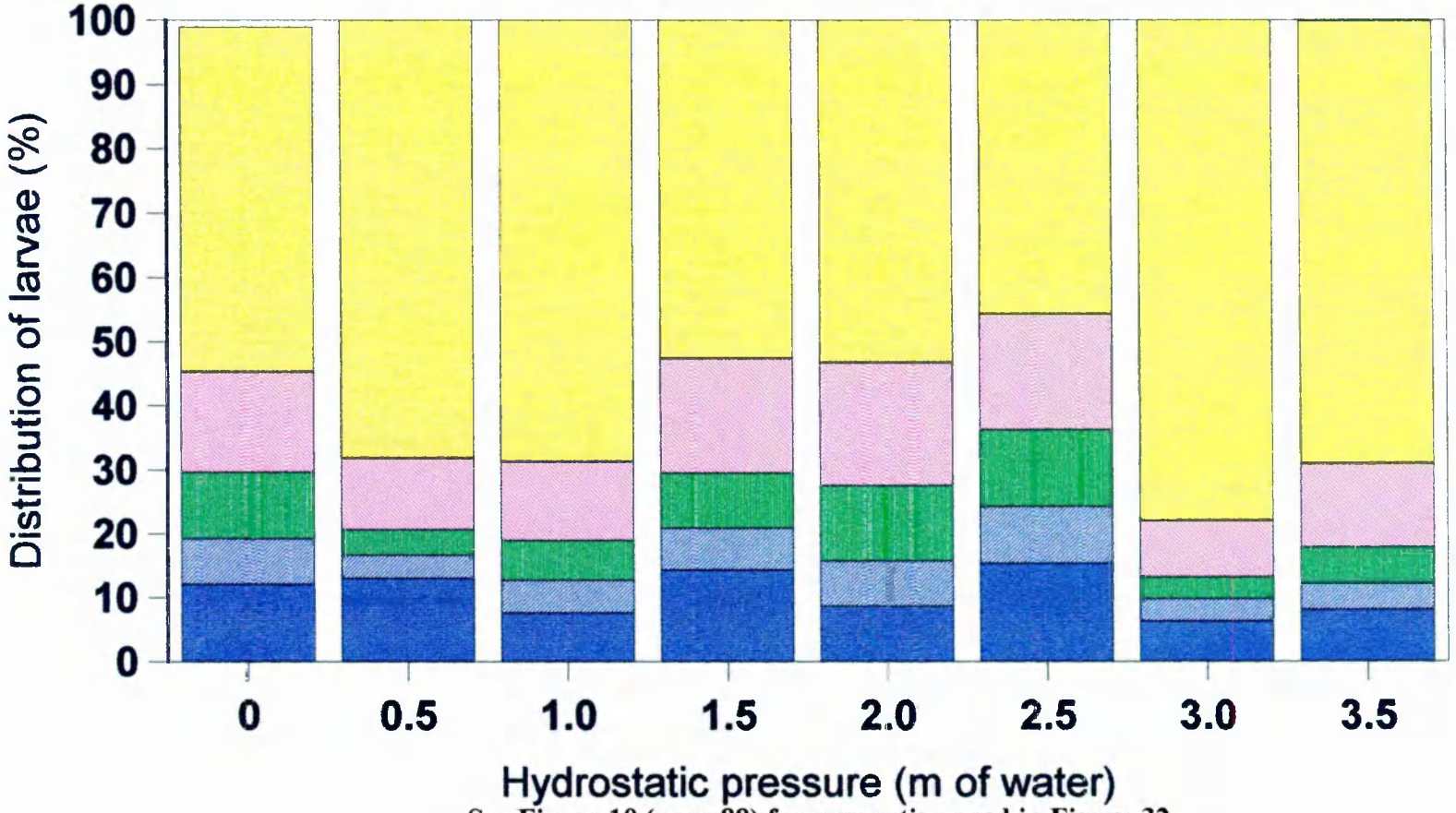
Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	*	ns	ns	ns	ns	ns
1.0	ns	**	-	ns	ns	**	**	*
1.5	ns	ns	*	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	*	ns	*	ns	ns	*	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 108 Distribution (and %) of mature *C. intestinalis* larvae in the vertical behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 67)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	153 (18.1)	3647 (56.7)	741 (45.6)	208 (44.4)	437 (58.3)	210 (40.6)	4249 (71.8)	803 (66.1)	322 (49.3)	3142 (72.4)	282 (64.4)	268 (40.0)	1028 (54.3)	738 (54.1)	576 (62.1)	542 (45.5)	349 (59.3)	280 (44.3)	1009 (42.9)	399 (66.3)	2180 (85.2)	731 (67.9)	717 (62.3)	4333 (70.7)	2472 (68.2)
Section B	194 (22.9)	928 (14.4)	325 (20.0)	65 (13.9)	116 (15.5)	98 (19.0)	592 (10.0)	195 (16.1)	111 (17.0)	461 (10.6)	67 (15.3)	110 (16.4)	362 (19.1)	288 (21.1)	136 (14.7)	248 (20.8)	76 (12.9)	104 (16.5)	462 (19.6)	63 (10.5)	183 (7.2)	126 (11.7)	157 (13.6)	706 (11.5)	569 (15.7)
Section C	198 (23.4)	660 (10.3)	192 (11.8)	34 (7.3)	55 (7.3)	52 (10.1)	182 (3.1)	88 (7.2)	86 (13.2)	212 (4.9)	48 (11.0)	53 (7.9)	158 (8.3)	171 (12.5)	99 (10.7)	137 (11.5)	71 (12.1)	59 (9.3)	299 (12.7)	43 (7.1)	51 (2.0)	48 (4.4)	96 (8.3)	365 (6.0)	153 (4.2)
Section D	147 (17.4)	453 (7.0)	115 (7.1)	37 (7.9)	51 (6.8)	45 (8.7)	166 (2.8)	63 (5.2)	56 (8.6)	197 (4.5)	17 (3.9)	63 (9.4)	116 (6.1)	90 (6.6)	60 (6.5)	96 (8.1)	42 (7.1)	70 (11.1)	206 (8.8)	37 (6.1)	47 (1.8)	63 (5.8)	63 (5.5)	266 (4.3)	122 (3.4)
Section E (bottom)	154 (18.2)	745 (11.6)	252 (11.5)	124 (26.5)	91 (12.1)	112 (21.7)	728 (12.3)	65 (5.4)	78 (11.9)	327 (7.5)	24 (5.5)	176 (26.3)	229 (12.1)	76 (5.6)	56 (6.0)	168 (14.1)	51 (8.7)	119 (18.8)	377 (16.0)	60 (10.0)	97 (3.8)	109 (10.1)	118 (10.3)	461 (7.5)	306 (8.4)
Number of larvae	846	6433	1625	468	750	517	5917	1214	653	4339	438	670	1893	1363	927	1191	589	632	2353	602	2558	1077	1151	6131	3622

Figure 32 Mean distributions of mature C. intestinalis larvae exposed to 1500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 32.

Table 109 Significances of differences in distributions (*G*-test) of *C. intestinalis* larvae with 1500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1		*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*		*	*	-														
	2	*	*		*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*		*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1		*	*	*	*	*	*	*	*	*	*	*	*		*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 110 Significances of differences in mean percentages (*t*-test) of *C. intestinalis* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	*	-	ns
3.5	*	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

The two-way ANOVA indicated that the proportion of larvae in the top section of the chamber varied significantly with both light intensity ($F_{(4, 80)} = 118.18$, $p<0.001$) and hydrostatic pressure ($F_{(7, 80)} = 5.598$, $p<0.001$), and that there was significant interaction between light intensity and hydrostatic pressure ($F_{(28, 80)} = 5.192$, $p<0.001$). The ANOVA also indicated that the proportion of larvae in the bottom section of the chamber varied significantly with both light intensity ($F_{(4, 80)} = 114.28$, $p<0.001$) and hydrostatic pressure ($F_{(7, 80)} = 9.075$, $p<0.001$), and that there was significant interaction between light intensity and hydrostatic pressure ($F_{(28, 80)} = 7.587$, $p<0.001$).

Table 111 ***C. intestinalis*** **two-way ANOVA summary table**

Section of chamber	Source	SS	df	Variance	F
Top	Between samples	17824.48	39		
	Factor a (light)	12819.37	4	3204.843	118.18
	Factor b (pressure)	1062.651	7	151.8073	5.59809
	Interaction	3942.455	28	140.80	5.192255
	Within samples	2169.415	80	27.11769	
Bottom	Between samples	14588.99	39		
	Factor a (light)	9097.146	4	2274.286	114.2836
	Factor b (pressure)	1264.107	7	180.5867	9.074540
	Interaction	4227.741	28	150.9908	7.587334
	Within samples	1592.03	80	19.90037	

A priori analysis of variance indicated that the percentages of larvae found in the top section of the vertical behaviour chamber, over the range of hydrostatic pressures, were significantly different ($F_{(1, 32)} > 9.57$) at each light intensity (Table 112). The percentages of larvae found in the bottom section of the chamber were, with the exception of those found

with light intensities of 0 and 1000 lux, significantly different ($F_{(1, 32)} > 5.77$) from each other (Table 112). No threshold effect could be detected in this range of light intensities.

Table 112 ***A priori*** **ANOVA of percentages of *C. intestinalis* larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values = $F_{1, 32}$)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light flux (lux)	0	250	500	1000	1500
0	-	35.89 ***	70.46 ***	3.209 ns	119.6 ***
250	9.57 **	-	5.775 *	60.57 ***	286.5 ***
500	40.4 ***	10.65 **	-	103.7 ***	373.6 ***
1000	11.03 **	41.16 ***	93.67 ***	-	83.59 ***
1500	186.7 ***	280.8 ***	400.8 ***	107.0 ***	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

A priori analysis of variance indicated that at hydrostatic pressures greater than 1.5 m head of water the percentages of larvae found in either the top or the bottom section of the vertical behaviour chamber, over the range of light intensities, were significantly different from each other in the majority of cases (Table 113). This hydrostatic pressure appears to be a threshold as larval behaviour changes as pressure increases beyond it.

Table 113 ***A priori*** **ANOVA of percentages of *C. intestinalis* larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values = $F_{1, 20}$)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	**	*	***	ns	ns
0.5	ns	-	ns	***	***	***	ns	ns
1.0	ns	ns	-	***	***	***	**	ns
1.5	*	**	**	-	ns	ns	*	***
2.0	*	**	***	ns	-	ns	ns	**
2.5	**	**	***	ns	ns	-	**	***
3.0	ns	ns	ns	*	*	*	-	ns
3.5	ns	ns	ns	*	**	**	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

12.3.2 *Ascidiella aspersa* larvae

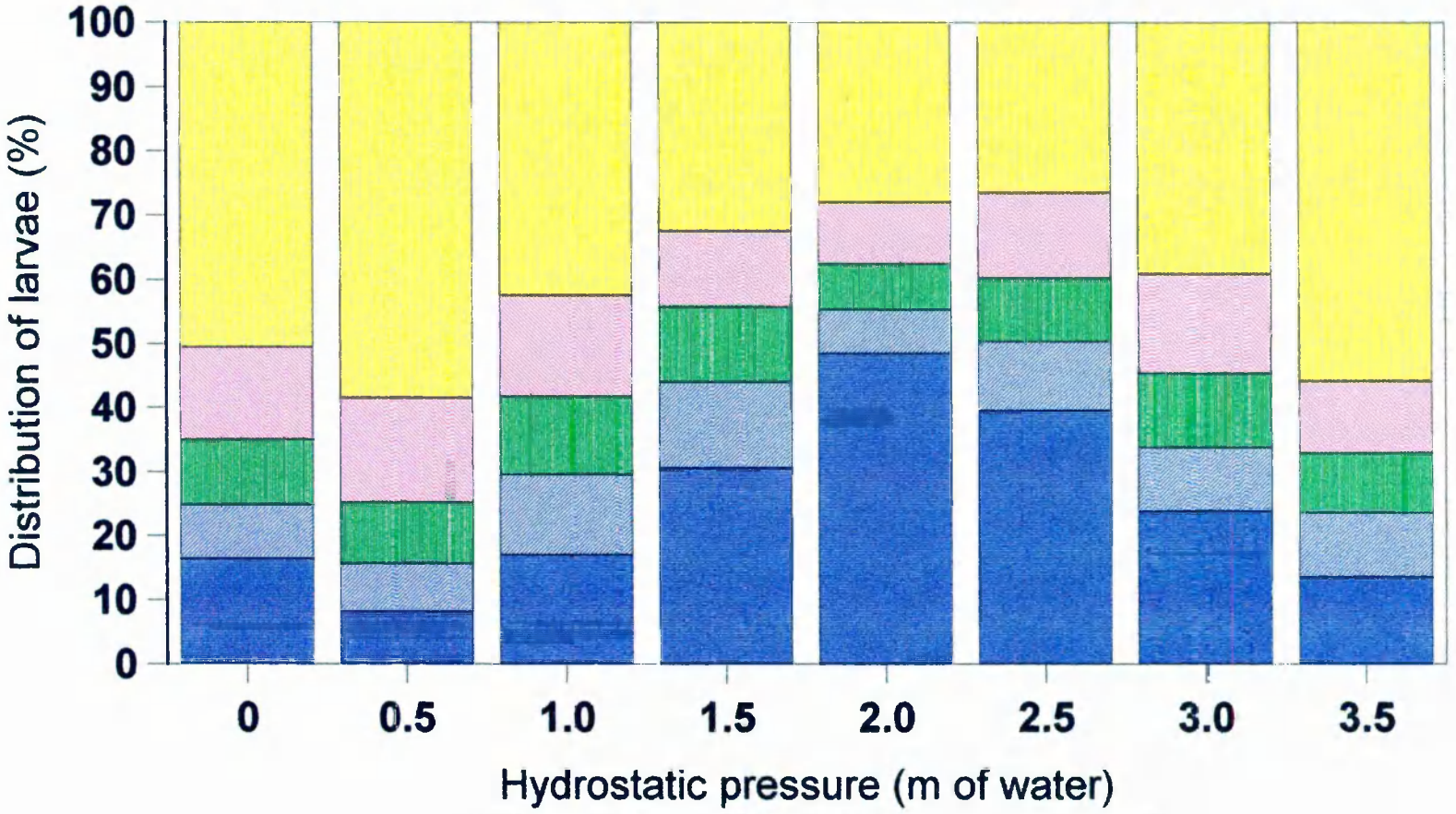
The distributions of *A. aspersa* larvae in the vertical behaviour chamber with a light flux of 250 lux intensity and hydrostatic pressures of 0 to 3.5 m head of water are presented in Table 114. In contrast to the maximum negative geotaxis observed at 2.0 m hydrostatic pressure in the absence of light (Figures 25 and 26), the mean larval distributions with light of 250 lux intensity show maximum negative phototaxis at 2.0 m hydrostatic pressure (Figure 33). The percentages of larvae in the top section of the chamber are generally greater with 250 lux light flux than in the absence of light, suggesting positive phototaxis; the maximum mean larval percentage in the top section occurred with an applied pressure of 0.5 m head of water. Although the majority of distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 115), most differences in the mean percentages of larvae in the top or bottom sections of the chamber are not significant ($p>0.05$, *t*-test); the majority of significant differences occur in the bottom section of the chamber (Table 116).

The distributions of *A. aspersa* larvae in the vertical behaviour chamber with a light flux of 500 lux intensity are presented in Table 117. The mean larval distributions are similar to those observed with 250 lux intensity, but the negative phototaxis observed at 2 m hydrostatic pressure has disappeared and proportions in the bottom section of the chamber are generally lower (Figure 34) indicating continued positive phototaxis, except at 0 m applied pressure where there is an apparent decrease in positive phototaxis. The maximum mean percentage in the top section occurs at 1.5 m hydrostatic pressure. Although the vast majority of larval distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 118), most of the differences in the mean percentages of larvae in the end sections of the chamber (Table 119) are not significantly different ($p>0.05$, *t*-test).

Table 114 Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 69)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	489 (16.1)	171 (45.4)	685 (51.8)	286 (50.7)	1158 (66.2)	319 (50.2)	221 (42.7)	202 (41.1)	142 (38.4)	252 (46.3)	243 (39.5)	137 (36.4)	188 (24.9)	226 (54.8)	104 (25.6)	446 (22.8)	136 (34.2)	132 (20.9)	81 (28.1)	216 (43.4)	311 (42.3)	162 (30.9)	270 (52.3)	303 (47.9)	487 (65.1)
Section B	708 (23.4)	46 (12.2)	205 (15.5)	78 (13.8)	245 (14.0)	139 (21.9)	90 (17.4)	72 (14.7)	59 (16.1)	91 (16.7)	69 (11.2)	48 (12.8)	90 (11.9)	47 (11.3)	22 (5.4)	198 (10.1)	49 (12.3)	82 (13.0)	44 (15.3)	74 (14.9)	87 (11.8)	112 (21.4)	65 (12.6)	78 (12.3)	69 (9.2)
Section C	679 (22.4)	34 (9.0)	140 (10.6)	57 (10.1)	141 (8.1)	67 (10.5)	71 (13.7)	55 (11.2)	47 (12.8)	69 (12.7)	71 (11.5)	43 (11.4)	92 (12.2)	26 (6.3)	4 (1.0)	170 (8.7)	35 (8.8)	71 (11.3)	25 (8.7)	51 (10.2)	68 (9.3)	84 (16.0)	48 (9.3)	70 (11.1)	61 (8.2)
Section D	566 (18.7)	42 (11.1)	105 (7.9)	41 (7.3)	112 (6.4)	41 (6.4)	61 (11.8)	62 (12.6)	52 (14.2)	61 (11.2)	84 (13.7)	35 (9.3)	113 (14.9)	25 (6.0)	20 (4.9)	142 (7.3)	44 (11.1)	65 (10.3)	33 (11.5)	63 (12.7)	62 (8.4)	49 (9.4)	58 (11.2)	95 (15.0)	35 (4.7)
Section E (bottom)	588 (19.4)	84 (22.3)	187 (14.1)	102 (18.1)	92 (5.3)	70 (11.0)	75 (14.5)	100 (20.4)	68 (18.5)	71 (13.1)	148 (24.1)	113 (30.1)	273 (36.1)	92 (22.1)	257 (63.1)	996 (51.0)	134 (33.7)	281 (44.5)	105 (36.5)	94 (18.9)	207 (28.2)	117 (22.3)	75 (14.5)	87 (13.7)	96 (12.8)
Number of larvae	3030	377	1322	564	1748	636	518	491	368	544	615	376	756	416	407	1952	398	631	288	498	735	524	516	633	748

Figure 33 Mean distributions of mature A. aspersa larvae exposed to 250 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 33.

Table 115 **Significances of differences in distributions (*G*-test) of *A. aspersa* larvae with 250 lux light flux and a variety of applied hydrostatic pressures**

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*		-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1		*	*	*	*	*	-																	
	2	*	*	*	*	*			-																
	3	*	*	*	*	*		*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*		*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*		*	*	*		*	-						
3.0	1		*	*	*	*	*			*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 116 **Significances of differences in mean percentages (*t*-test) of *A. aspersa* larvae in section A (top) and section E (bottom) of the behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

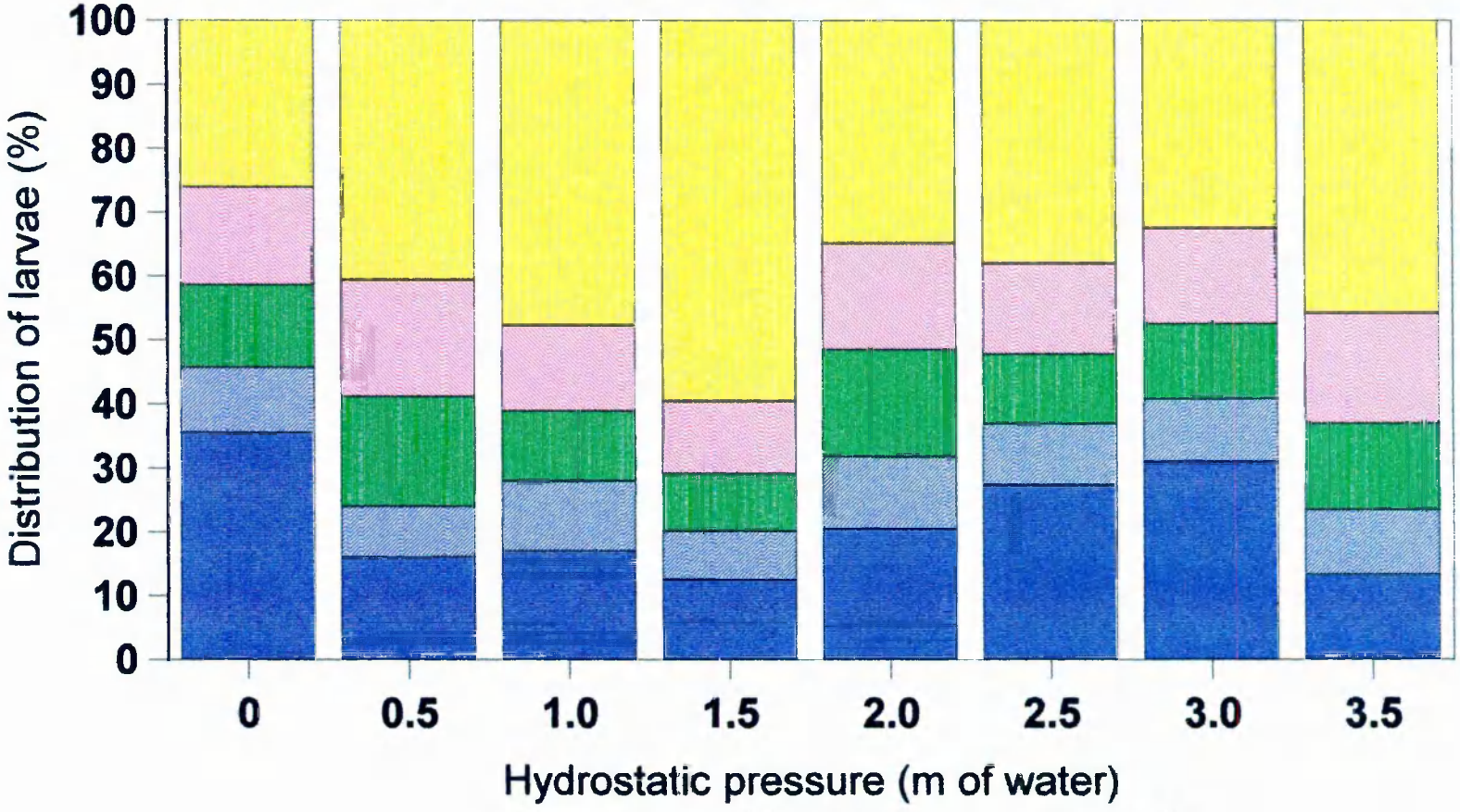
Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	*	ns	**	ns	ns
0.5	ns	-	ns	*	ns	**	*	ns
1.0	ns	ns	-	*	ns	**	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	*
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	*	*	ns	ns	ns	-	*	*
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	*	ns	*	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 117 **Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures**

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 70)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	489 (16.1)	172 (30.3)	58 (35.8)	321 (23.3)	244 (30.3)	190 (51.8)	672 (43.3)	233 (49.7)	296 (47.4)	281 (46.4)	217 (49.4)	117 (38.4)	932 (67.3)	63 (38.2)	346 (36.3)	82 (28.5)	102 (41.5)	281 (38.3)	150 (36.1)	106 (57.6)	340 (25.7)	274 (38.8)	179 (38.6)	402 (51.9)	189 (43.2)
Section B	708 (23.4)	93 (16.4)	24 (14.8)	205 (14.9)	199 (24.7)	41 (11.2)	255 (16.4)	54 (11.5)	86 (13.8)	88 (14.5)	63 (14.4)	79 (25.9)	101 (7.3)	29 (17.6)	174 (18.3)	31 (10.8)	45 (18.3)	82 (11.2)	69 (16.6)	28 (15.2)	199 (15.0)	105 (14.9)	77 (16.6)	136 (17.6)	73 (16.7)
Section C	679 (22.4)	80 (14.1)	28 (17.3)	165 (12.0)	219 (27.2)	41 (11.2)	208 (13.4)	55 (11.7)	64 (10.3)	66 (10.9)	45 (10.3)	60 (19.7)	84 (6.1)	38 (23.0)	148 (15.5)	48 (16.7)	27 (11.0)	85 (11.6)	40 (9.6)	28 (15.2)	141 (10.6)	91 (12.9)	73 (15.7)	95 (12.3)	58 (13.2)
Section D	566 (18.7)	73 (12.9)	22 (13.6)	120 (8.7)	31 (3.9)	34 (9.3)	151 (9.7)	46 (9.8)	65 (10.4)	76 (12.5)	42 (9.6)	23 (7.5)	99 (7.2)	21 (12.7)	118 (12.4)	18 (6.3)	27 (11.0)	87 (11.9)	18 (4.3)	10 (5.4)	127 (9.6)	80 (11.3)	54 (11.6)	81 (10.5)	36 (8.2)
Section E (bottom)	588 (19.4)	150 (26.4)	30 (18.5)	569 (41.2)	112 (13.9)	61 (16.6)	266 (17.1)	81 (17.3)	113 (18.1)	95 (15.7)	72 (16.4)	26 (8.5)	168 (12.1)	14 (8.5)	166 (17.4)	109 (37.8)	45 (18.3)	198 (27.0)	139 (33.4)	12 (6.5)	518 (39.1)	156 (22.1)	81 (17.5)	60 (7.8)	82 (18.7)
Number of larvae	3030	568	162	1380	805	367	1552	469	624	606	439	305	1384	165	952	288	246	733	416	184	1325	706	464	774	438

Figure 34 Mean distributions of mature A. aspersa larvae exposed to 500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 34.

Table 118 Significances of differences in distributions (*G*-test) of *A. aspersa* larvae with 500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2		-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*		*	-																	
	2	*	*	*	*		*		-																
	3	*	*	*	*	*	*	*		-															
1.5	1	*	*	*	*						-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*		*				*	*	*		*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*		*	*	*		*	*	*	*	*	*		*	*	*	*	*	*		-			
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	3	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*		*	-	

□= not significant (p>0.05); * = p<0.05.

Table 119 Significances of differences in mean percentages (*t*-test) of *A. aspersa* larvae in section A (top) and section E (bottom) of the behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	*	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	*	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	*	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

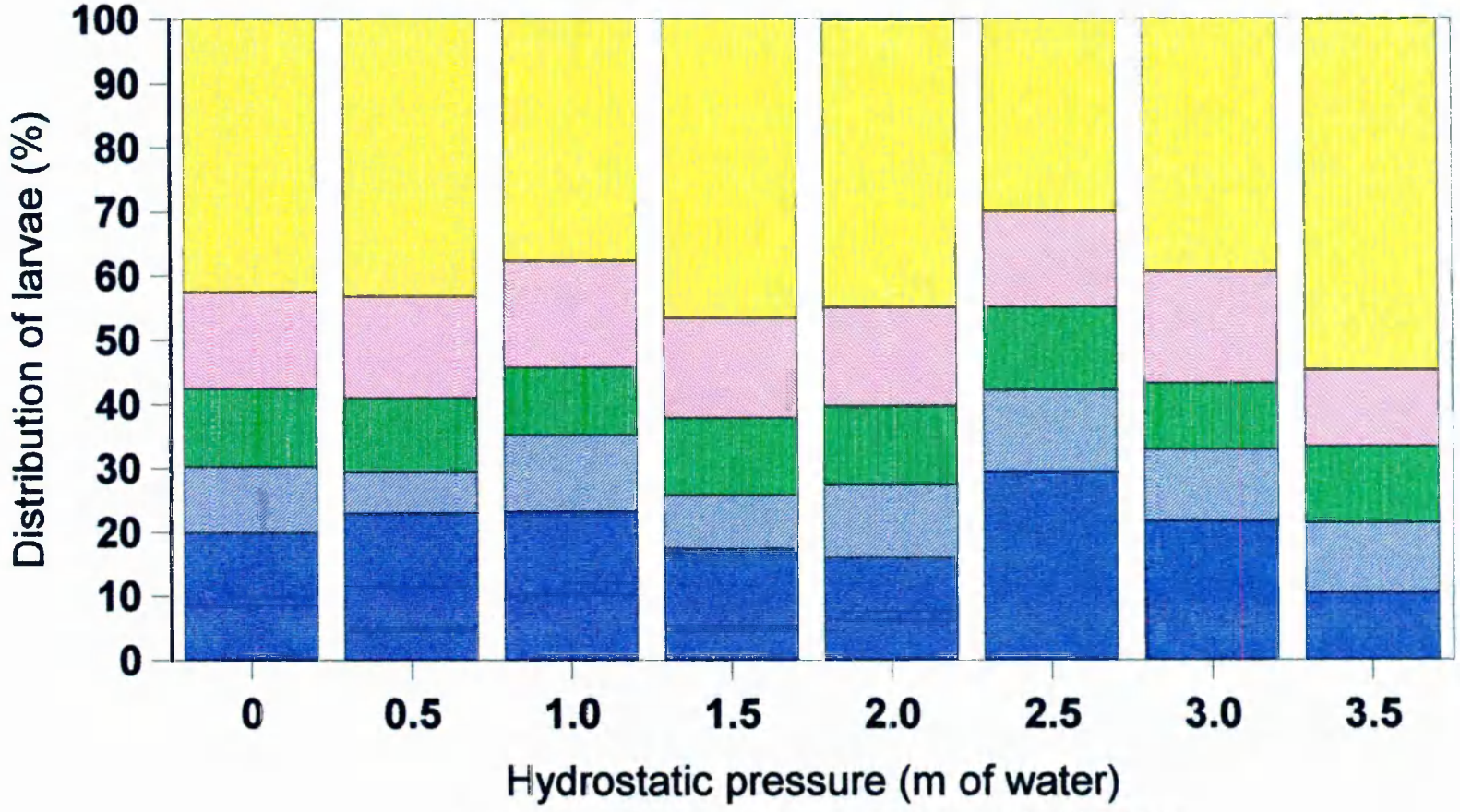
The distributions of *A. aspersa* larvae in the vertical behaviour chamber with a light flux of 1000 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 120. Compared with the distributions observed with 250 and 500 lux, the proportion of larvae in the bottom section of the chamber continued to decline, but the tendency for larvae to accumulate in the upper sections at pressures around 1.5 m head of water was less pronounced than was observed with 500 lux. Nevertheless, as pressure is increased, the mean percentage of larvae observed in the bottom section of the chamber reaches a minimum at 2.0 m hydrostatic pressure (Figure 35); at pressures greater than 2.0 m head of water the proportion of larvae in the top section increases, and that in the bottom section decreases, establishing a potential circulation cell. Although the majority of larval distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 121), few of the differences in the mean percentages of larvae in the end sections of the chamber are significant (Table 122).

The distributions of *A. aspersa* larvae in the vertical behaviour chamber with a light flux of 1500 lux intensity are presented in Table 123. The mean larval distributions from 0 m to 2.0 m hydrostatic pressure are similar to those observed with 1000 lux, but the minimum percentage of larvae in the bottom section of the chamber was observed with 1.5 m head of water (Figure 35). In contrast to the potential circulation-cell distribution noted at hydrostatic pressures greater than 2.5 m with a light intensity of 1000 lux, when a light intensity of 1500 lux was applied the proportion of larvae in the bottom section of the chamber increased at hydrostatic pressures greater than 2.0 m. Despite the majority of larval distributions being significantly different ($p<0.05$, *G*-test) from each other (Table 124), few of the differences in the mean percentages of larvae in the end sections of the chamber are significant (Table 125).

Table 120 Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 71)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	167 (31.1)	437 (54.5)	173 (35.5)	129 (46.4)	1022 (38.1)	889 (50.7)	159 (41.2)	93 (57.1)	351 (33.5)	158 (36.2)	224 (40.4)	481 (55.9)	113 (40.9)	76 (51.7)	149 (45.0)	54 (18.6)	80 (24.0)	236 (38.4)	130 (33.8)	33 (26.6)	436 (43.2)	311 (48.9)	1041 (64.1)	153 (31.3)
Section B	150 (17.6)	68 (12.7)	127 (15.8)	81 (16.6)	46 (16.5)	415 (15.5)	282 (16.1)	66 (17.1)	32 (19.6)	167 (15.9)	70 (16.0)	79 (14.3)	139 (16.2)	45 (16.3)	16 (10.9)	55 (16.6)	39 (13.4)	58 (17.4)	88 (14.3)	63 (16.4)	21 (16.9)	181 (17.9)	103 (16.2)	115 (7.1)	109 (22.3)
Section C	148 (17.4)	90 (16.8)	83 (10.3)	49 (10.0)	35 (12.6)	211 (7.9)	299 (17.0)	37 (9.6)	21 (12.9)	110 (10.5)	50 (11.4)	56 (10.1)	116 (13.5)	29 (10.5)	17 (11.6)	47 (14.2)	41 (14.1)	39 (11.7)	81 (13.2)	39 (10.1)	21 (16.9)	98 (9.7)	87 (13.7)	163 (10.0)	77 (15.7)
Section D	196 (23.0)	92 (17.1)	62 (7.7)	34 (7.0)	31 (11.2)	162 (6.0)	111 (6.3)	42 (10.9)	13 (8.0)	136 (13.0)	49 (11.2)	70 (12.6)	35 (4.1)	31 (11.2)	20 (13.6)	36 (10.9)	52 (17.9)	39 (11.7)	66 (10.7)	47 (12.2)	18 (14.5)	103 (10.2)	78 (12.3)	154 (9.5)	68 (13.9)
Section E (bottom)	182 (21.3)	120 (22.3)	93 (11.6)	151 (30.9)	37 (13.3)	873 (32.5)	174 (9.9)	82 (21.2)	4 (2.5)	284 (27.1)	110 (25.2)	125 (22.6)	89 (10.3)	58 (21.0)	18 (12.2)	44 (13.3)	104 (35.9)	117 (35.1)	143 (23.3)	106 (27.5)	31 (25.0)	192 (19.0)	57 (9.0)	151 (9.3)	82 (16.8)
Number of larvae	853	537	802	488	278	2683	1755	386	163	1048	437	554	860	276	147	331	290	333	614	385	124	1010	636	1624	489

Figure 35 Mean distributions of mature A. aspersa larvae exposed to 1000 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 35.

Table 121 Significances of differences in distributions (*G*-test) of *A. aspersa* larvae with 1000 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*		*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*		*		-														
	2	*	*	*	*	*	*		*	*		-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*		*				*	-											
	2	*		*		*	*	*	*	*	*	*	*	*	-										
	3	*	*	*		*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*			*		*	*	*	*	-							
3.0	1	*	*	*	*	*	*	*	*			*	*	*	*	*	*	*	-						
	2		*	*	*	*	*	*	*			*	*	*	*	*	*			-					
	3	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	-					
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	

□= not significant (p>0.05); * = p<0.05.

Table 122 Significances of differences in mean percentages (*t*-test) of *A. aspersa* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

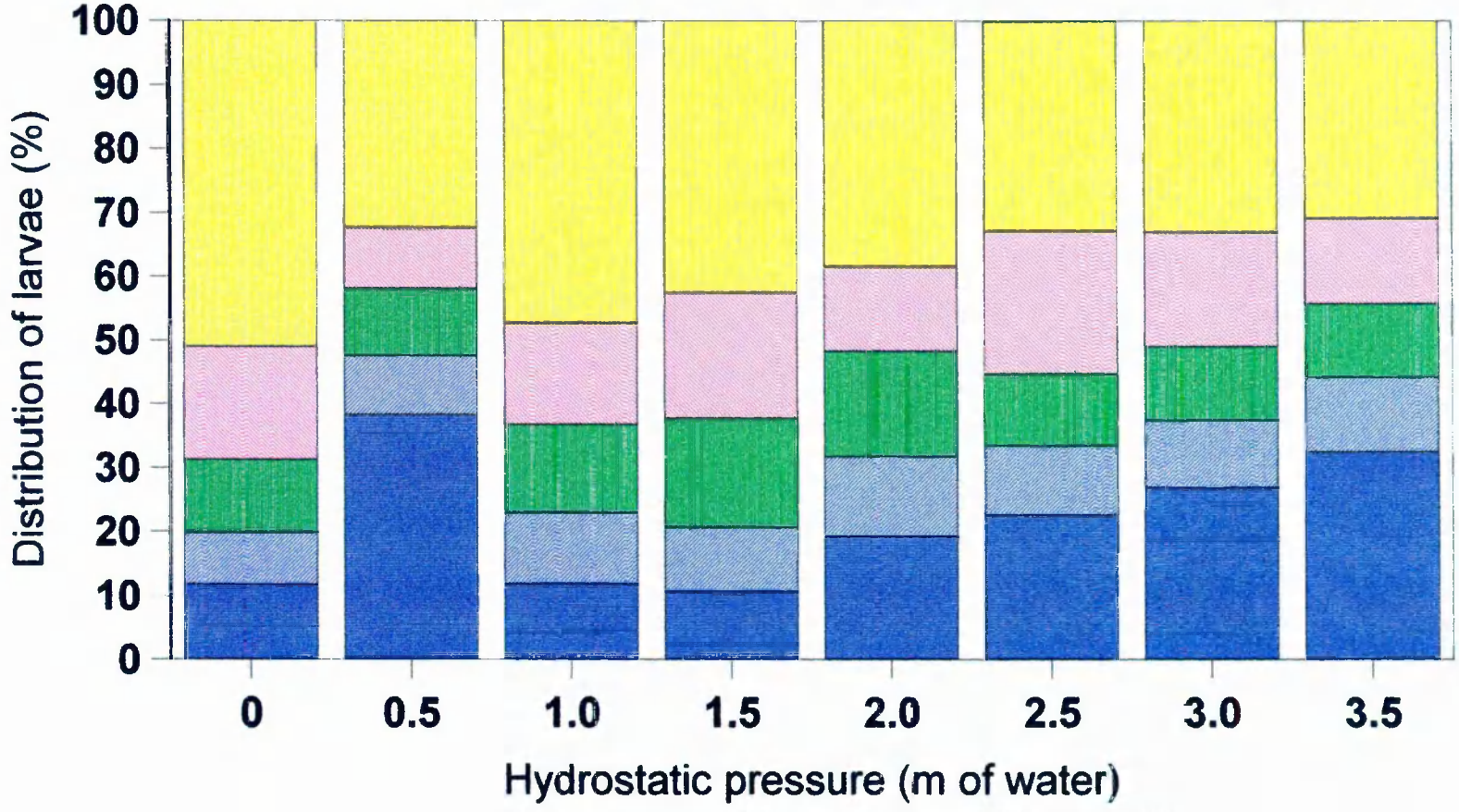
Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	*	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	*
3.0	ns	ns	ns	ns	ns	ns	-	*
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 123 Distribution (and %) of mature *A. aspersa* larvae in the vertical behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 72)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	173 (41.8)	578 (59.4)	193 (41.5)	1102 (29.4)	373 (36.7)	431 (38.2)	442 (47.6)	946 (43.2)	580 (55.4)	643 (44.8)	440 (39.6)	102 (43.0)	140 (36.3)	294 (36.7)	317 (41.8)	118 (32.2)	280 (44.3)	122 (20.7)	175 (35.4)	149 (28.1)	225 (35.3)	112 (37.0)	170 (28.2)	295 (30.7)
Section B	150 (17.6)	70 (16.9)	186 (19.1)	73 (15.7)	173 (4.6)	176 (17.3)	216 (19.1)	142 (15.3)	387 (17.7)	133 (12.7)	283 (19.7)	226 (20.4)	40 (16.9)	61 (15.8)	99 (12.4)	98 (12.9)	51 (13.9)	104 (16.5)	201 (34.2)	102 (20.6)	132 (24.9)	65 (10.2)	35 (11.6)	72 (12.0)	145 (15.1)
Section C	148 (17.4)	73 (17.6)	83 (8.5)	56 (12.0)	349 (9.3)	151 (14.9)	127 (11.2)	140 (15.1)	326 (14.9)	110 (10.5)	255 (17.8)	177 (15.9)	44 (18.6)	53 (13.7)	146 (18.2)	122 (16.1)	39 (10.7)	59 (9.3)	82 (13.9)	53 (10.7)	64 (12.1)	74 (11.6)	29 (9.6)	89 (14.8)	97 (10.1)
Section D	196 (23.0)	54 (13.0)	52 (5.3)	43 (9.2)	275 (7.3)	129 (12.7)	136 (12.0)	69 (7.4)	311 (14.2)	82 (7.8)	121 (8.4)	123 (11.1)	32 (13.5)	36 (9.3)	77 (9.6)	129 (17.0)	47 (12.8)	70 (11.1)	55 (9.4)	50 (10.1)	63 (11.9)	62 (9.7)	31 (10.2)	56 (9.3)	131 (13.6)
Section E (bottom)	182 (21.3)	44 (10.6)	74 (7.6)	100 (21.5)	1854 (49.4)	187 (18.4)	219 (19.4)	135 (14.5)	219 (10.0)	142 (13.6)	134 (9.3)	144 (13.0)	19 (8.0)	96 (24.9)	186 (23.2)	93 (12.3)	111 (30.3)	119 (18.8)	128 (21.8)	114 (23.1)	122 (23.0)	211 (33.1)	96 (31.7)	215 (35.7)	293 (30.5)
Number of larvae	853	414	973	465	3753	1016	1129	928	2189	1047	1436	1110	237	386	802	759	366	632	588	494	530	637	303	602	961

Figure 36 Mean distributions of mature A. aspersa larvae exposed to 1500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 36.

Table 124 Significances of differences in distributions (*G*-test) of *A. aspersa* larvae with 1500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3		*	*	*	*	*	*		*		*	-												
2.0	1	*	*		*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*		-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 125 Significances of differences in mean percentages (*t*-test) of *A. aspersa* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	*	-	ns	ns	ns	*	***
1.5	ns	ns	ns	-	ns	*	*	***
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	*	*	ns	ns	-	ns
3.5	ns	ns	*	*	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

The two-way ANOVA indicated that the proportion of larvae in the top section of the chamber varied significantly with light intensity ($F_{(4, 80)} = 88.21$, $p<0.001$) and hydrostatic pressure ($F_{(7, 80)} = 10.071$, $p<0.001$), and that there was significant interaction between light and hydrostatic pressure ($F_{(28, 80)} = 8.021$, $p<0.001$). The ANOVA also indicated that the proportion of larvae in the bottom section of the chamber varied significantly with light intensity ($F_{(4, 80)} = 20.60$, $p<0.001$) and hydrostatic pressure ($F_{(7, 80)} = 2.321$, $p<0.05$), and that there was significant interaction between light and hydrostatic pressure ($F_{(28, 80)} = 2.748$, $p<0.001$). The results for the top and bottom sections of the chamber are summarised in Table 126.

Table 126 ***A. aspersa*** **two-way ANOVA summary table**

Section of chamber	Source	SS	df	Variance	F
Top	Between samples	5826.347	39		
	Factor a (light)	3172.805	4	793.2013	88.21
	Factor b (pressure)	633.8917	7	90.55596	10.0705
	Interaction	2019.65	28	72.13	8.021436
	Within samples	719.3761	80	8.992202	
Bottom	Between samples	6406.77	39		
	Factor a (light)	3002.012	4	750.503	20.60
	Factor b (pressure)	591.7422	7	84.5346	2.32051
	Interaction	2813.015	28	100.46	2.757801
	Within samples	29.14.346	80	36.42933	

A priori analysis of variance indicated that the percentages of larvae found, over the range of pressures, in the top section of the vertical behaviour chamber in the absence of light were significantly different ($F_{(1, 32)} > 41.8$) to the percentages observed at all other light

intensities, and the percentages of larvae found in the bottom section of the vertical behaviour chamber in the absence of light were significantly different ($F_{(1, 32)} > 32.2$) to the percentages observed at all other light intensities (Table 127).

Table 127 ***A priori*** **ANOVA of percentages of** ***A. aspersa*** **larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values = $F_{1, 32}$)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light flux (lux)	0	250	500	1000	1500
0	-	32.23 ***	55.78 ***	56.15 ***	52.74 ***
250	65.17 ***	-	3.208 ns	3.298 ns	2.511 ns
500	62.51 ***	0.028 ns	-	0.001 ns	0.043 ns
1000	61.88 ***	0.043 ns	0.002 ns	-	0.054 ns
1500	41.78 ***	2.588 ns	2.081 ns	1.966 ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

A priori analysis of variance indicated that at a pressure of 2.5 m head of water the percentages of larvae found in both the top and bottom sections of the vertical behaviour chamber, over the range of light intensities, were significantly different from those found with most other applied pressures (Table 128). This hydrostatic pressure appears to be a threshold as larval behaviour changes when the pressure is changed.

Table 128 ***A priori*** **ANOVA of percentages of** ***A. aspersa*** **larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values = $F_{1, 20}$)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	*	ns	ns
1.0	ns	ns	-	ns	ns	**	ns	ns
1.5	ns	ns	ns	-	ns	**	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	*	**	**	*	*	-	ns	*
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	**	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

12.3.3 *Styela clava* larvae

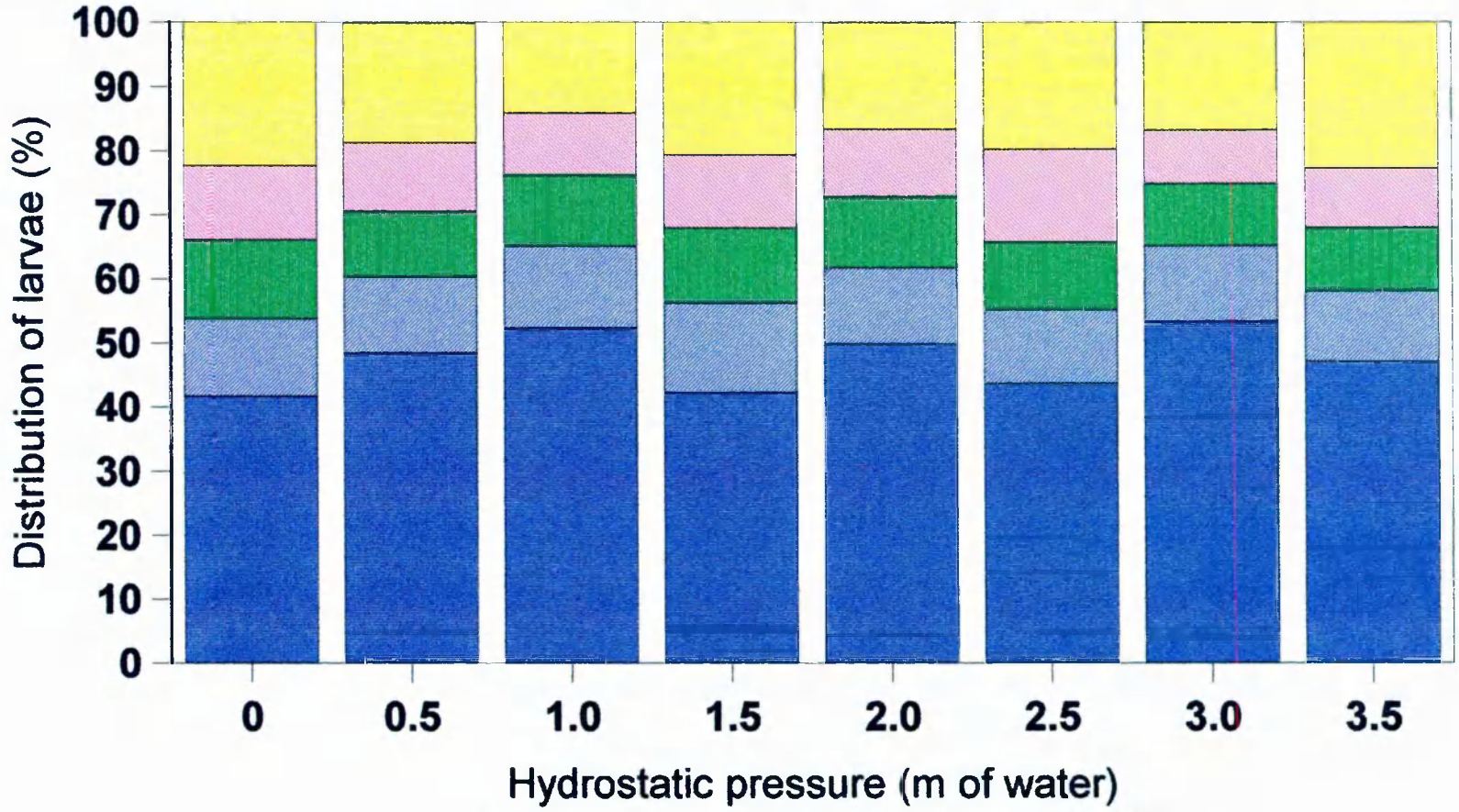
The distributions of *S. clava* larvae in the vertical behaviour chamber with a light flux of 250 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 129. In contrast to the negative geotaxis exhibited in the absence of light (Figures 27 and 28) the mean distributions of *S. clava* larvae with a light flux of 250 lux show increased proportions of larvae in the bottom section of the chamber (Figure 37) which suggests either cessation of negative geotaxis, with passive sinking, or active negative phototaxis. The majority of distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 130), but none of the differences in the mean percentages of larvae in the top or bottom sections of the chamber are significant (Table 131).

The distributions of *S. clava* larvae in the vertical behaviour chamber with a light flux of intensity 500 lux and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 132. The mean larval distributions are markedly different to those observed with 250 lux with large proportions of larvae congregating in the top section of the chamber (Figure 38). The proportions of larvae in the top section are greater than those recorded in the absence of light, suggesting a positive phototactic response; maximum response was observed at 1.5 m hydrostatic pressure, with a gradual decline in response as the pressure increased and decreased. The vast majority of larval distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 133), and many of the differences in the mean percentages of larvae in the end sections of the chamber are significant ($p<0.05$, *t*-test). The majority of significant differences between the mean percentages of larvae found in either the top or bottom sections of the chamber occur at applied pressures greater than 2 m head of water (Table 134).

Table 129 Distribution (and %) of mature *S. clava* larvae in the vertical behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 74)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	26 (22.0)	712 (20.4)	339 (28.3)	103 (14.1)	193 (26.5)	38 (11.4)	174 (24.3)	13 (7.7)	890 (13.3)	217 (22.6)	72 (18.4)	72 (18.6)	88 (18.2)	159 (14.9)	270 (17.5)	37 (23.6)	169 (30.7)	46 (8.2)	380 (18.2)	170 (16.6)	290 (15.3)	91 (14.3)	23 (9.5)	392 (29.6)
Section B	150 (17.6)	22 (18.6)	368 (10.5)	163 (13.6)	71 (9.7)	60 (8.2)	62 (18.7)	52 (7.3)	23 (13.6)	671 (10.0)	97 (10.1)	56 (14.3)	45 (11.6)	76 (15.7)	108 (10.1)	143 (9.2)	15 (9.6)	105 (19.1)	63 (11.2)	198 (9.5)	91 (8.9)	137 (7.2)	47 (7.4)	22 (9.1)	133 (10.0)
Section C	148 (17.4)	22 (18.6)	413 (11.8)	160 (13.4)	66 (9.0)	84 (11.5)	33 (9.9)	63 (8.8)	43 (25.4)	726 (10.8)	82 (8.6)	66 (16.8)	55 (14.2)	69 (14.3)	103 (9.6)	169 (10.9)	19 (12.1)	66 (12.0)	49 (8.7)	211 (10.1)	99 (9.7)	169 (8.9)	59 (9.3)	18 (7.5)	141 (10.6)
Section D	196 (23.0)	11 (9.3)	403 (11.6)	161 (13.5)	93 (12.7)	74 (10.2)	47 (14.2)	131 (18.3)	33 (19.5)	805 (12.0)	118 (12.3)	69 (17.6)	57 (14.7)	88 (18.2)	130 (12.2)	152 (9.8)	17 (10.8)	42 (7.6)	86 (15.3)	294 (14.1)	116 (11.3)	180 (9.5)	73 (11.5)	15 (6.2)	154 (11.6)
Section E (bottom)	182 (21.3)	37 (31.4)	1593 (45.7)	374 (31.2)	399 (54.5)	317 (43.5)	152 (45.8)	296 (41.3)	57 (33.7)	3613 (53.9)	445 (46.4)	129 (32.9)	159 (41.0)	163 (33.7)	568 (53.2)	812 (52.5)	69 (43.9)	168 (30.5)	317 (56.5)	1003 (48.1)	548 (53.5)	1116 (59.0)	366 (57.5)	163 (67.6)	506 (38.2)
Number of larvae	853	118	3489	1197	732	728	332	716	169	6705	959	392	388	484	1068	1546	157	550	561	2086	1024	1892	636	241	1326

Figure 37 Mean distributions of mature S. clava larvae exposed to 250 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 37.

Table 130 Significances of differences in distributions (*G*-test) of *S. clava* larvae with 250 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*		*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*		*	-											
	2	*	*	*		*	*	*	*		*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*		*	*		*		*	*		*		*	*		-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*		*	*	*	*	*	*	*	*	*			*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*		*	*	*	*		*	*	*	*		*	*	*	*	*			-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 131 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in section A (top) and section E (bottom) of the behaviour chamber with 250 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

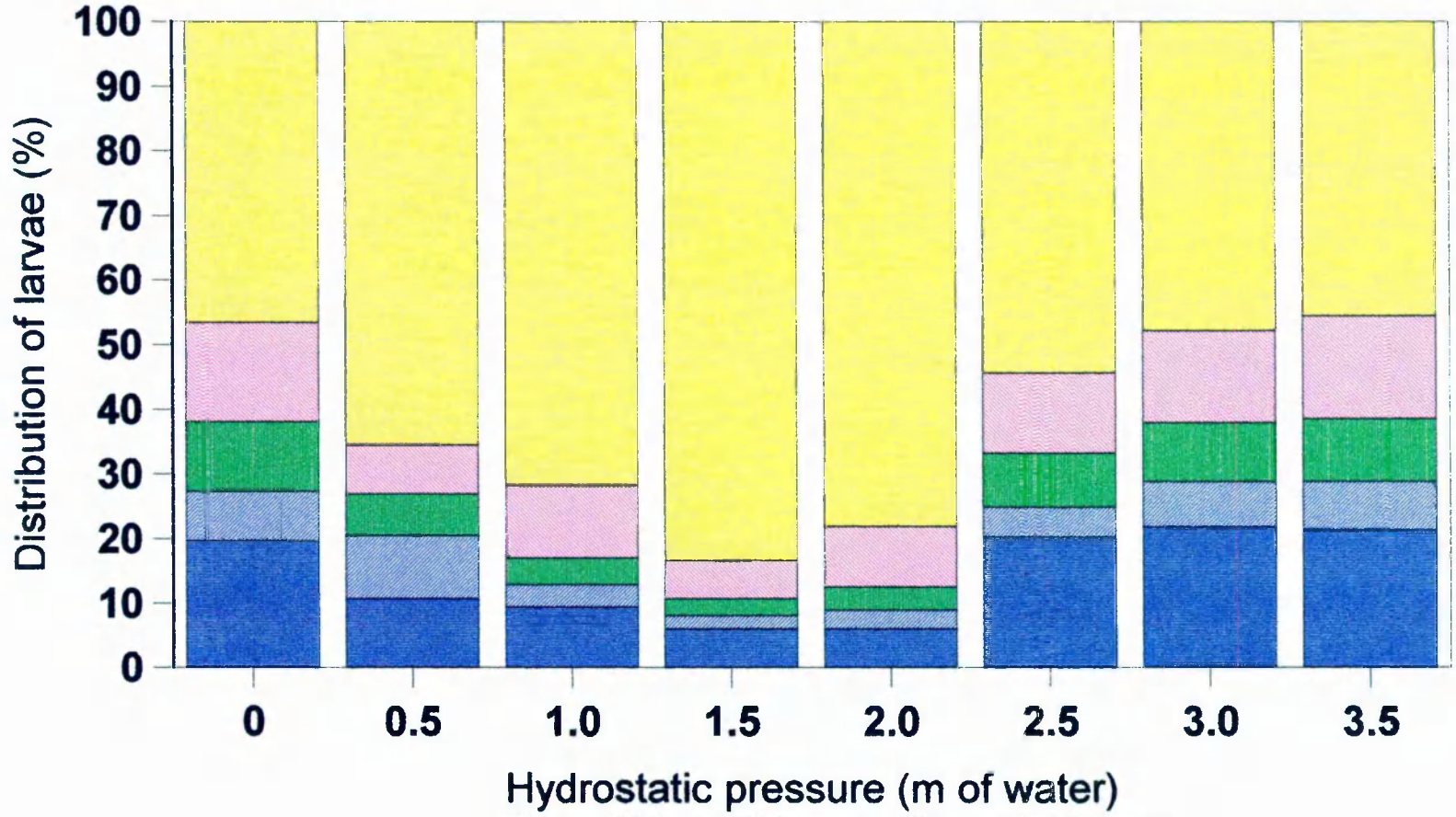
Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	ns	ns
2.0	ns	ns	ns	ns	-	ns	ns	ns
2.5	ns	ns	ns	ns	ns	-	ns	ns
3.0	ns	ns	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 132 Distribution (and %) of mature *S. clava* larvae in the vertical behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 75)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	519 (45.8)	184 (44.1)	550 (48.4)	60 (40.0)	2242 (66.7)	194 (64.7)	1452 (71.1)	186 (85.3)	405 (68.5)	985 (86.9)	3965 (82.1)	828 (85.4)	988 (72.6)	471 (69.2)	5184 (80.2)	425 (56.4)	191 (46.2)	366 (57.3)	204 (46.6)	419 (41.3)	800 (52.7)	547 (48.6)	198 (32.1)	392 (52.5)
Section B	150 (17.6)	137 (12.1)	61 (14.6)	214 (18.8)	19 (12.7)	236 (7.0)	35 (11.7)	272 (13.3)	6 (2.8)	44 (7.4)	33 (2.9)	324 (6.7)	52 (5.4)	141 (10.4)	74 (10.9)	581 (9.0)	89 (11.8)	60 (14.5)	75 (11.7)	78 (17.8)	152 (15.0)	192 (12.6)	185 (16.4)	98 (15.9)	112 (15.0)
Section C	148 (17.4)	117 (10.3)	45 (10.8)	128 (11.3)	7 (4.7)	215 (6.4)	22 (7.3)	100 (4.9)	7 (3.2)	11 (1.9)	32 (2.8)	135 (2.8)	15 (1.5)	65 (4.8)	26 (3.8)	214 (3.3)	62 (8.2)	41 (9.9)	47 (7.4)	39 (8.9)	120 (11.8)	111 (7.3)	97 (8.6)	69 (11.2)	76 (10.2)
Section D	196 (23.0)	76 (6.7)	55 (13.2)	76 (6.7)	12 (8.0)	345 (10.3)	16 (5.3)	59 (2.9)	5 (2.3)	36 (6.1)	16 (1.4)	101 (2.1)	27 (2.8)	41 (3.0)	37 (5.4)	171 (2.6)	39 (5.2)	24 (5.8)	21 (3.3)	26 (5.9)	99 (9.8)	82 (5.4)	71 (6.3)	66 (10.7)	46 (6.2)
Section E (bottom)	182 (21.3)	285 (25.1)	72 (17.3)	169 (14.9)	52 (34.7)	323 (9.6)	33 (11.0)	158 (7.7)	14 (6.4)	95 (16.1)	68 (6.0)	304 (6.3)	47 (4.9)	125 (9.2)	73 (10.7)	315 (4.9)	138 (18.3)	97 (23.5)	130 (20.3)	91 (20.8)	224 (22.1)	333 (21.9)	226 (20.1)	185 (30.0)	121 (16.2)
Number of larvae	853	1134	417	1137	150	3361	300	2041	218	591	1134	4829	969	1360	681	6465	753	413	639	438	1014	1518	1126	616	747

Figure 38 Mean distributions of mature S. clava larvae exposed to 500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 38.

Table 133 Significances of differences in distributions (*G*-test) of *S. clava* larvae with 500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1	*	*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*	*	*	*		*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 134 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in section A (top) and section E (bottom) of the behaviour chamber with 500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	*	*	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	*	ns	-	ns	ns	ns	ns	ns
1.5	***	ns	ns	-	ns	**	***	*
2.0	*	ns	ns	ns	-	*	*	*
2.5	ns	ns	*	**	*	-	ns	ns
3.0	ns	ns	*	**	**	ns	-	ns
3.5	ns	ns	*	*	*	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

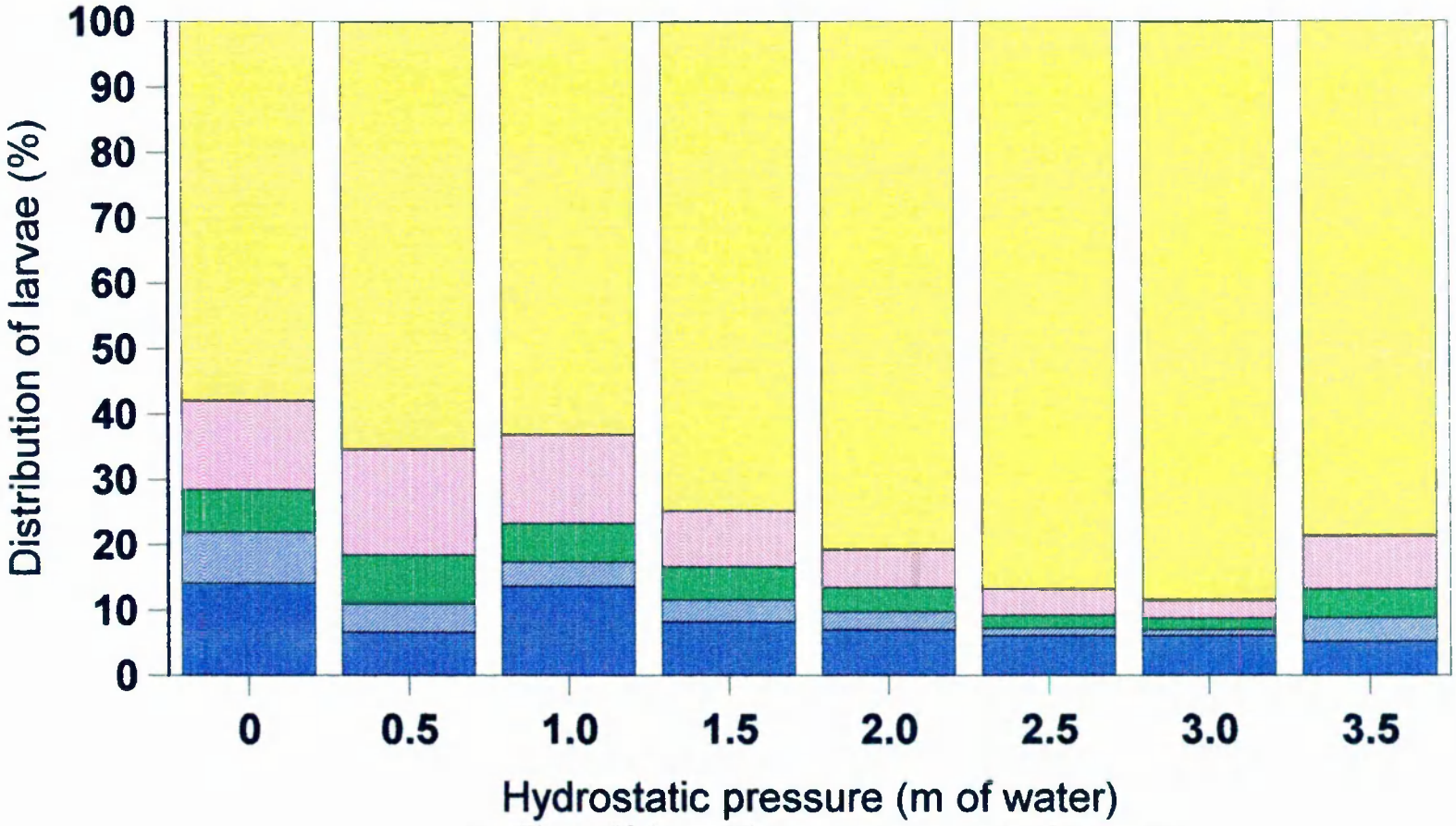
The distributions of *S. clava* larvae in the vertical behaviour chamber with a light flux of 1000 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 135. The mean larval distributions are similar to those observed with 500 lux, but with larger proportions of larvae found in the top section of the chamber, particularly at hydrostatic pressures greater than 2.0 m head of water (Figure 39) This indicates continued positive phototaxis. The maximum positive phototactic response was observed at 3.0 m hydrostatic pressure. The majority of larval distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 136), and many of the differences in the mean percentages of larvae in the end sections of the chamber are significant ($p<0.05$, *t*-test); the majority of significant differences between mean larval percentages in the end sections of the chamber are found at applied pressures greater than 2 m head of water (Table 137).

The distributions of *S. clava* larvae in the vertical behaviour chamber with a light flux of 1500 lux intensity and applied hydrostatic pressures ranging from 0 to 3.5 m head of water are presented in Table 138. The mean larval distributions are similar to those observed with 1000 lux but with smaller proportions of larvae found in the top section of the chamber, particularly at hydrostatic pressures less than 1.0 m head of water (Figure 40), suggesting attenuation of the positive phototactic response. The maximum positive phototactic response was observed at 2.5 m hydrostatic pressure. The majority of larval distributions are significantly different ($p<0.05$, *G*-test) from each other (Table 139), but not many of the differences in the mean percentages of larvae in the end sections of the chamber are significant ($p<0.05$, *t*-test). All of the significant differences between mean larval percentages are found in the top section of the chamber (Table 140).

Table 135 Distribution (and %) of mature *S. clava* larvae in the vertical behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 76)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	367 (51.5)	447 (67.5)	580 (56.1)	481 (54.4)	1861 (61.0)	3817 (69.4)	489 (66.3)	59 (27.1)	332 (75.8)	538 (70.2)	2428 (76.7)	306 (68.6)	599 (81.5)	1362 (80.8)	444 (79.6)	2301 (83.0)	4546 (86.1)	4624 (89.5)	1489 (85.2)	606 (83.8)	4310 (90.3)	805 (79.9)	1123 (75.2)	2346 (80.1)
Section B	150 (17.6)	126 (17.7)	89 (13.4)	115 (11.1)	158 (17.9)	504 (16.5)	871 (15.8)	119 (16.1)	37 (17.0)	33 (7.5)	93 (12.1)	225 (7.1)	59 (13.2)	58 (7.9)	60 (3.6)	54 (9.7)	119 (4.3)	216 (4.1)	191 (3.7)	72 (41)	30 (4.1)	102 (2.1)	87 (8.6)	176 (11.8)	175 (6.0)
Section C	148 (17.4)	61 (8.6)	39 (5.9)	57 (5.5)	70 (7.9)	356 (11.7)	275 (5.0)	41 (5.5)	20 (9.2)	22 (5.0)	38 (5.0)	155 (4.9)	30 (6.7)	29 (3.9)	53 (3.1)	29 (5.2)	106 (3.8)	100 (1.9)	62 (1.2)	65 (3.7)	28 (3.9)	33 (0.7)	44 (4.4)	73 (4.9)	123 (4.2)
Section D	196 (23.0)	47 (6.6)	22 (3.3)	118 (11.4)	57 (6.4)	161 (5.3)	196 (3.6)	29 (3.9)	16 (7.3)	7 (1.6)	27 (3.5)	105 (3.3)	14 (3.1)	16 (2.2)	61 (3.6)	4 (0.7)	68 (2.5)	42 (0.8)	35 (0.7)	28 (1.6)	14 (1.9)	24 (0.5)	49 (4.9)	51 (3.4)	93 (3.2)
Section E (bottom)	182 (21.3)	111 (15.6)	65 (9.8)	164 (15.9)	119 (13.4)	167 (5.5)	341 (6.2)	60 (8.1)	86 (39.4)	44 (10.0)	70 (9.1)	253 (8.0)	37 (8.3)	33 (4.5)	149 (8.8)	27 (4.8)	179 (6.5)	374 (7.1)	256 (5.0)	94 (5.4)	45 (6.2)	305 (6.4)	23 (2.3)	70 (4.7)	192 (6.6)
Number of larvae	853	712	662	1034	885	3049	5500	738	218	438	766	3166	446	735	1685	558	2773	5278	5168	1748	723	4774	1008	1493	2929

Figure 39 Mean distributions of mature S. clava larvae exposed to 1000 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 39.

Table 136 Significances of differences in distributions (*G*-test) of *S. clava* larvae with 1000 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2	*	-																						
	3	*	*	-																					
0.5	1		*	*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*		*	*	*		-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*		*	*	*	*	*	*	*	-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*		*	*	*			*	*		*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*		-					
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-				
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-		
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	

□= not significant (p>0.05); * = p<0.05.

Table 137 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1000 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

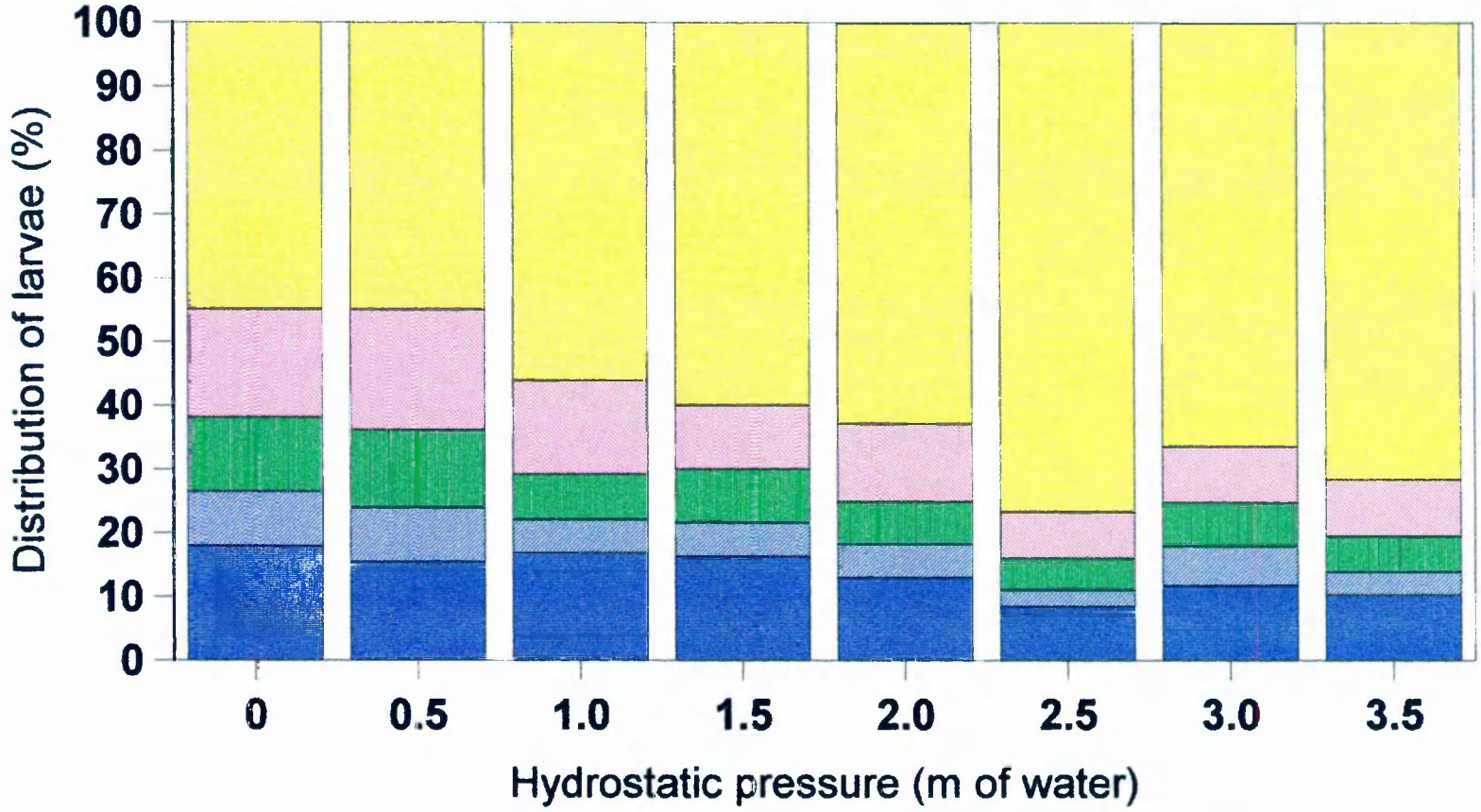
Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	*	*	ns	*
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	ns	ns	ns	-	ns	ns	**	ns
2.0	*	*	ns	ns	-	ns	ns	ns
2.5	*	*	ns	*	ns	-	ns	ns
3.0	*	*	ns	*	ns	ns	-	ns
3.5	*	*	ns	ns	ns	*	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

Table 138 **Distribution (and %) of mature *S. clava* larvae in the vertical behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures**

		Hydrostatic pressure (m head of water)																							
	Control	0 (Table 77)			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
Section A (top)	177 (20.8)	374 (45.8)	184 (48.8)	278 (41.7)	261 (50.4)	691 (54.5)	303 (30.3)	370 (59.3)	81 (37.3)	756 (57.8)	276 (57.0)	527 (60.9)	384 (61.4)	1471 (62.9)	2031 (60.4)	2132 (65.4)	620 (71.1)	4722 (77.7)	228 (74.8)	151 (71.6)	677 (70.8)	3493 (65.4)	624 (70.3)	461 (66.4)	1818 (73.7)
Section B	150 (17.6)	115 (14.1)	64 (17.0)	138 (20.7)	81 (15.6)	214 (16.9)	231 (23.1)	55 (8.8)	46 (21.2)	214 (16.4)	69 (14.3)	107 (12.4)	21 (3.4)	362 (15.5)	324 (9.6)	408 (12.5)	85 (9.7)	416 (6.8)	25 (8.2)	17 (8.1)	85 (8.9)	467 (8.7)	80 (9.0)	73 (10.5)	202 (8.2)
Section C	148 (17.4)	86 (10.5)	39 (10.3)	92 (13.8)	49 (9.5)	124 (9.8)	167 (16.7)	40 (6.4)	24 (11.1)	89 (6.8)	43 (8.9)	70 (8.1)	53 (8.5)	166 (7.1)	252 (7.5)	182 (5.6)	49 (5.6)	303 (5.0)	10 (3.3)	10 (4.7)	50 (5.2)	388 (7.3)	52 (5.9)	49 (7.1)	130 (5.3)
Section D	196 (23.0)	62 (7.6)	27 (7.2)	67 (10.0)	30 (5.8)	103 (8.1)	100 (10.0)	31 (5.0)	13 (6.0)	67 (5.1)	33 (6.8)	42 (4.8)	29 (4.6)	136 (5.8)	171 (5.1)	163 (5.0)	27 (3.1)	141 (2.3)	10 (3.3)	7 (3.3)	32 (3.3)	365 (6.8)	26 (2.9)	36 (5.2)	84 (3.4)
Section E (bottom)	182 (21.3)	179 (21.9)	63 (16.7)	92 (13.8)	97 (18.7)	136 (10.7)	198 (19.8)	128 (20.5)	53 (24.4)	182 (13.9)	63 (13.0)	120 (13.9)	138 (22.1)	204 (8.7)	587 (17.4)	377 (11.6)	91 (10.4)	494 (8.1)	32 (10.5)	26 (12.3)	112 (11.7)	626 (11.7)	105 (11.8)	75 (10.8)	234 (9.5)
Number of larvae	853	816	377	667	518	1268	999	624	217	1308	484	866	625	2339	3365	3262	872	6076	305	211	956	5339	887	694	2468

Figure 40 Mean distributions of mature S. clava larvae exposed to 1500 lux light flux at a variety of hydrostatic pressures



See Figure 10 (page 88) for convention used in Figure 40.

Table 139 Significances of differences in distributions (*G*-test) of *S. clava* larvae with 1500 lux light flux and a variety of applied hydrostatic pressures

Applied pressure (m of water)		0			0.5			1.0			1.5			2.0			2.5			3.0			3.5		
	Exp	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	1	-																							
	2		-																						
	3	*	*	-																					
0.5	1			*	-																				
	2	*	*	*	*	-																			
	3	*	*	*	*	*	-																		
1.0	1	*	*	*	*	*	*	-																	
	2	*	*	*	*	*	*	*	-																
	3	*	*	*	*	*	*	*	*	-															
1.5	1	*	*	*	*		*	*	*		-														
	2	*	*	*	*	*	*	*	*	*	*	-													
	3	*	*	*	*	*	*	*	*	*	*	*	-												
2.0	1	*	*	*	*	*	*	*	*	*	*	*	*	-											
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	-										
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-									
2.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-								
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-							
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			-						
3.0	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*					-					
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*		-				
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-			
3.5	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*			*	-		
	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*		*	-	
	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-

□= not significant (p>0.05); * = p<0.05.

Table 140 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in section A (top) and section E (bottom) of the behaviour chamber with 1500 lux light flux and a variety of applied hydrostatic pressures

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Applied pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	ns	ns	ns	ns	ns
1.5	**	ns	ns	-	ns	ns	ns	ns
2.0	**	ns	ns	ns	-	ns	ns	ns
2.5	***	ns	ns	***	**	-	ns	ns
3.0	**	ns	ns	*	ns	ns	-	ns
3.5	**	ns	ns	*	ns	ns	ns	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

The two-way ANOVA indicated that the proportion of larvae in the top section of the chamber varied significantly with light intensity ($F_{(4, 80)} = 155.80$, $p<0.001$) and hydrostatic pressure ($F_{(7, 80)} = 4.389$, $p<0.001$), and that there was significant interaction between light and hydrostatic pressure ($F_{(28, 80)} = 5.280$, $p<0.001$). The ANOVA also indicated that the proportion of larvae in the bottom section of the chamber varied significantly with light intensity ($F_{(4, 80)} = 97.21$, $p<0.001$) but not with hydrostatic pressure ($F_{(7, 80)} = 1.375$, $p>0.05$), and that there was significant interaction between light and hydrostatic pressure ($F_{(28, 80)} = 2.319$, $p<0.01$). The results for the top and bottom sections of the chamber are summarised in Table 141.

Table 141 ***S. clava*** **two-way ANOVA summary table**

Section of chamber	Source	SS	df	Variance	F
Top	Between samples	201.87.96	39		
	Factor a (light)	15691.91	4	3922.978	155.8
	Factor b (pressure)	773.5648	7	110.5093	4.388763
	Interaction	3722.486	28	132.95	5.279813
	Within samples	2014.404	80	25.18004	
Bottom	Between samples	11044.35	39		
	Factor a (light)	9267.413	4	2316.853	97.21
	Factor b (pressure)	229.3994	7	32.77134	1.375078
	Interaction	1547.543	28	55.27	2.319091
	Within samples	1906.588	80	23.83235	

A priori analysis of variance indicated that the percentages of larvae found, over the range of hydrostatic pressures, in the top section of the vertical behaviour chamber in the absence of light were, with one exception, significantly different ($F_{(1, 32)} > 15.83$) at all light

intensities tested (Table 142). The majority of the percentages of larvae found in the bottom section of the vertical chamber were also significantly different ($F_{(1, 2)} > 13.70$).

Table 142 ***A priori*** **ANOVA of percentages of** ***S. clava*** **larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values =** $F_{1, 32}$**)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light flux (lux)	0	250	500	1000	1500
0	-	174.1 ***	0.708 ns	25.14 ***	1.724 ns
250	188.4 ***	-	197.0 ***	331.6 ***	210.5 ***
500	18.83 ***	326.4 ***	-	17.41 ***	0.222 ns
1000	92.03 ***	543.8 ***	27.60 ***	-	13.70 ***
1500	15.83 ***	313.5 ***	0.130 ns	31.52 ***	-

ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

A priori analysis of variance indicated that at hydrostatic pressures between 1.5 m and 3.0 m head of water the percentages of larvae found in the top section of the vertical behaviour chamber, over the range of light intensities, were significantly different from each other in the majority of cases (Table 143). This suggests that 1.5 m hydrostatic pressure is a threshold as larval behaviour changes as pressure increases beyond it.

Table 143 ***A priori*** **ANOVA of percentages of** ***S. clava*** **larvae in top (section A) and bottom (section E) of the vertical behaviour chamber (values =** $F_{1, 20}$**)**

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Pressure (m head of water)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
0	-	ns	ns	ns	ns	ns	ns	ns
0.5	ns	-	ns	ns	ns	ns	ns	ns
1.0	ns	ns	-	*	ns	ns	ns	ns
1.5	**	**	*	-	ns	ns	ns	ns
2.0	**	**	*	ns	-	ns	ns	ns
2.5	**	**	*	ns	ns	-	ns	ns
3.0	**	*	ns	ns	ns	ns	-	ns
3.5	ns	ns	ns	ns	ns	ns	ns	-

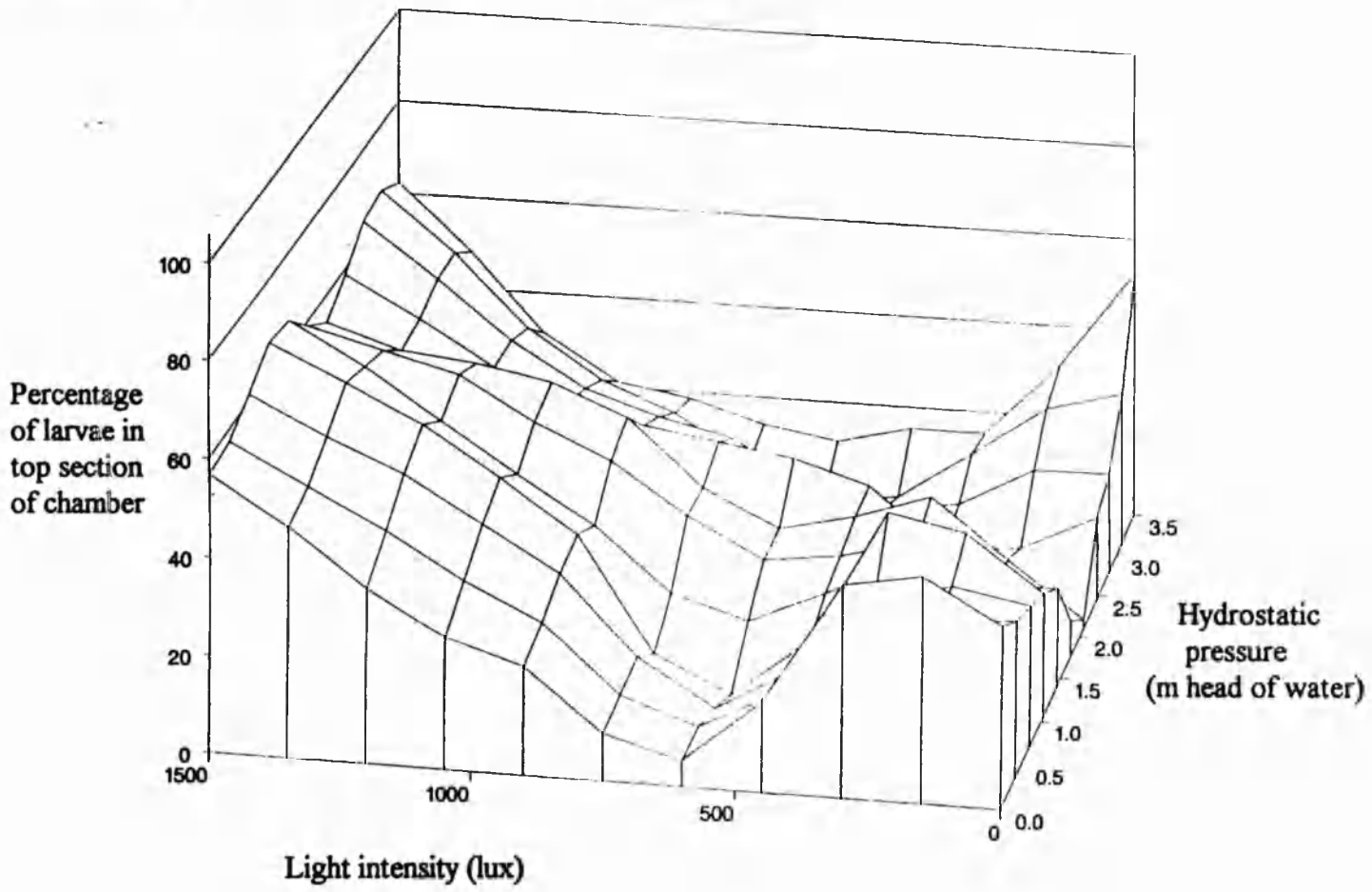
ns = not significant (p>0.05); * = p<0.05; ** = p<0.01; *** = p<0.001.

12.4 Discussion

When exposed to a light flux of 250 lux *Ciona intestinalis* larvae exhibited a similar, but attenuated, variation in distribution with hydrostatic pressure to that observed in the absence of light, indicating that light at this intensity has little effect. The variation in larval distribution with hydrostatic pressure was eliminated when light intensity was increased to 500 lux, with approximately 10% of larvae accumulating in the top section of the chamber. An increase in the light flux to 1000 lux produced an increase in the proportion of larvae in the top section of the chamber to approximately 30%. Further increasing the light flux to 1500 lux increased the proportion of larvae in the top section of the chamber to over 50%. Increased upward movement of larvae with increased light intensity must be an active phototactic response; this response is displayed at light fluxes greater than 1000 lux.

The variation in the proportion of the population of *C. intestinalis* larvae found in the top section of the vertical behaviour chamber under the range of light intensities and hydrostatic pressures employed in these experiments is presented as a three-dimensional surface graph in Figure 41. The graph can be viewed as a "landscape of distribution" which indicates the variation in proportion of the population accumulating in the top 20 cm section of a 1 m column of water under various combinations of light and hydrostatic pressure. The graph indicates that larvae hatched in light levels above 500 lux will tend to rise in the water column. If light levels near the surface decline the larvae will sink, and in so doing they will be exposed to increased hydrostatic pressure and reduced light intensity. As hydrostatic pressure increases to 2.5 m and light intensity decreases the larvae will tend to rise again, particularly at light intensities around 500 lux, and a circulation cell will be formed on the side of the "valley".

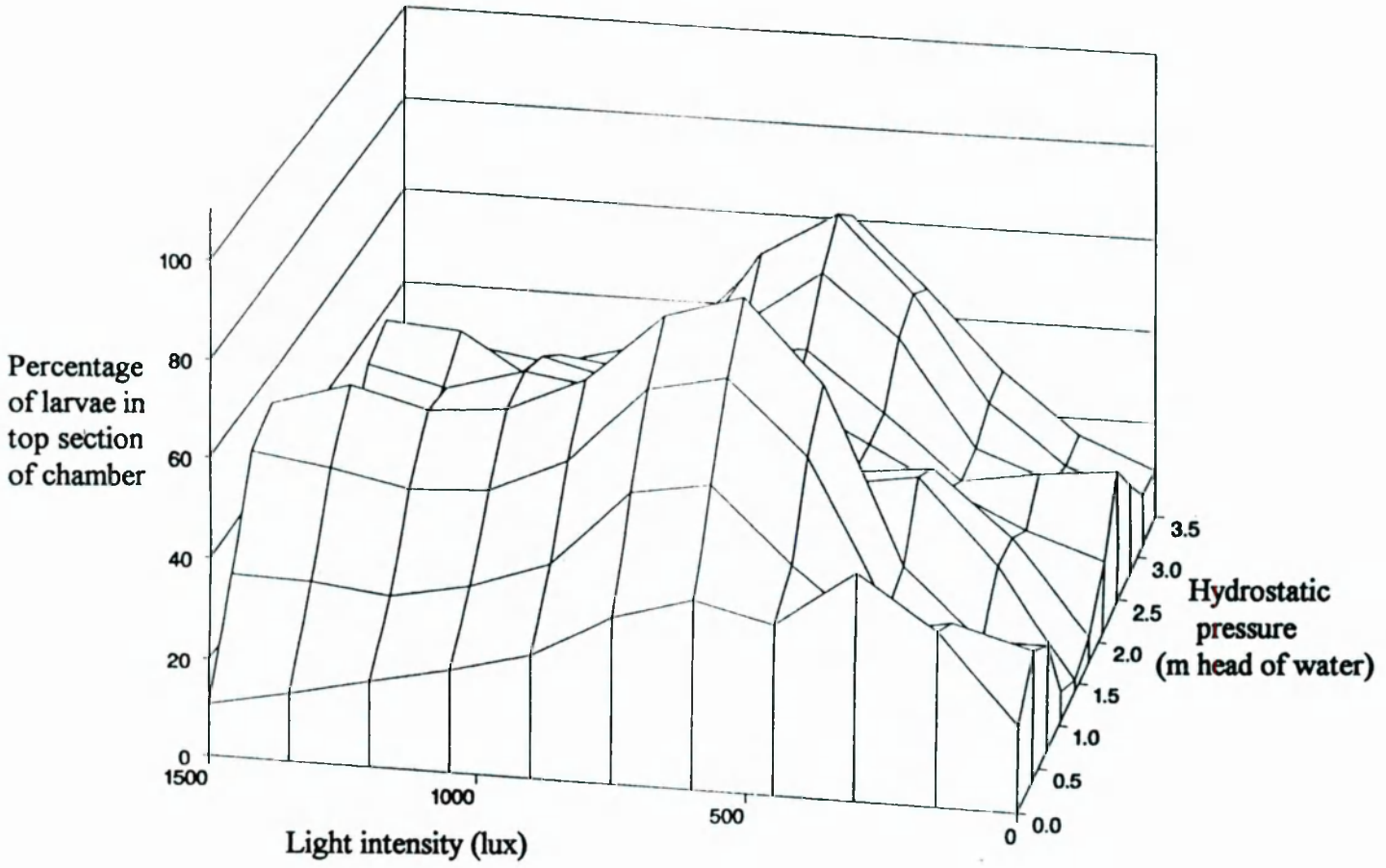
FIGURE 41 THE PROPORTION OF *C. INTESTINALIS* LARVAE IN THE TOP SECTION OF THE VERTICAL BEHAVIOUR CHAMBER UNDER THE RANGE OF LIGHT INTENSITIES AND HYDROSTATIC PRESSURES EMPLOYED THROUGHOUT THIS STUDY



The proportions of *Ascidiella aspersa* larvae in the top section of the chamber were generally greater with a light flux 250 lux intensity than in the absence of light, suggesting that positive phototaxis is enhancing the negative geotaxis observed in the absence of light. In contrast to the distributions recorded in the absence of light, the maximum proportion of larvae was observed in the bottom section of the chamber at 2 m hydrostatic pressure; at pressures greater than 2 m head of water the proportion of larvae in the top section of the chamber increased with applied pressure. A light flux of 500 lux intensity produced larval distributions similar to those observed with 250 lux, but the proportions of larvae in the bottom section of the chamber were generally lower indicating continued positive phototaxis. The proportion of larvae in the bottom section of the chamber declined further when the light intensity was increased to 1000 lux, with a minimum at 2.0 m hydrostatic pressure; at hydrostatic pressures greater than 2.0 m the proportion of larvae in the top section increased. Similar larval distributions were found at hydrostatic pressures between 0 and 2.0 m with a light flux of 1500 lux intensity, but at pressures greater than 2.0 m head of water the proportion of larvae in the bottom section of the chamber increased.

The variation in the proportion of the population of *A. aspersa* larvae found in the top section of the vertical chamber is presented in Figure 42. Larvae hatched at depth in low light will tend to rise in the water column; in so doing they will be exposed to reduced pressure and increased light intensity and, at a hydrostatic pressure of about 2 m, they will tend to sink again to start a new cycle. Larvae that escape from this cycle will rise in the water column; in so doing they will be exposed to reduced pressure and increased light intensity and will tend to sink again to rejoin the circulation cell. A large proportion of the larvae are, in effect, trapped in the "valley between the two ridges" forming an extended larval circulation cell.

FIGURE 42 THE PROPORTION OF *A. ASPERSA* LARVAE IN THE TOP SECTION OF THE VERTICAL BEHAVIOUR CHAMBER UNDER THE RANGE OF LIGHT INTENSITIES AND HYDROSTATIC PRESSURES EMPLOYED THROUGHOUT THIS STUDY



A second larval circulation cell is formed by larvae close to the surface; as these larvae sink they experience increased pressure and reduced light intensity, stimuli which elicit upward motion into a zone of reduced pressure and high light intensity where the cycle re-commences.

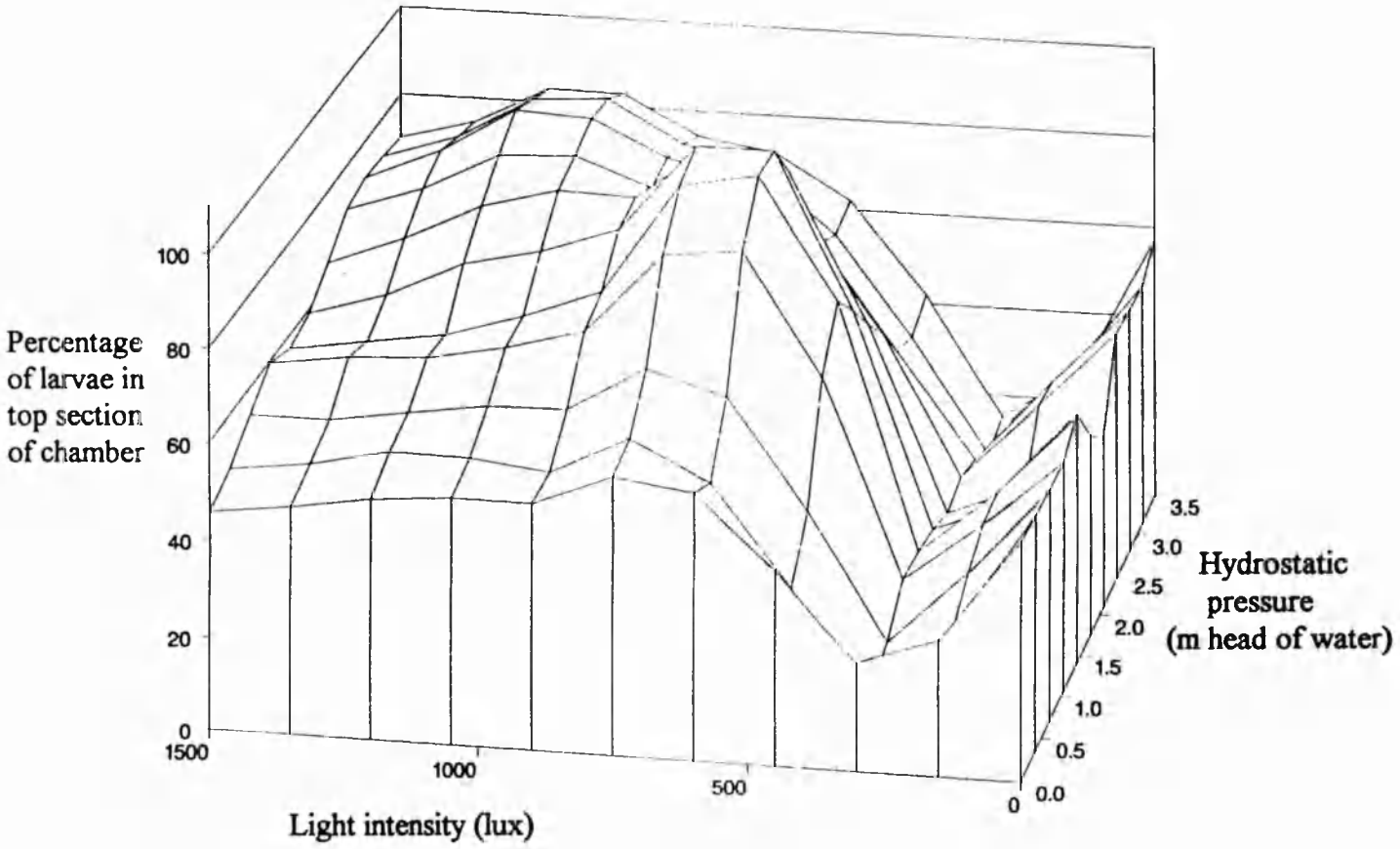
In contrast to the negative geotaxis exhibited by *Styela clava* in the absence of light, the distributions of larvae exposed to light flux of 250 lux intensity showed that the larvae accumulated in the bottom section of the chamber; this indicates either cessation of negative geotaxis, with passive sinking, or active negative phototaxis. The larval distributions changed markedly when a light flux of 500 lux intensity was applied, with large proportions of larvae congregating in the top section of the chamber. As the proportions of larvae in the top section exceeded those recorded in the absence of light, this re-distribution indicates a positive phototactic response; maximum response was observed at 1.5 m hydrostatic pressure. Similar larval distributions to those recorded with a light flux of 500 lux intensity were observed with 1000 lux intensity but with larger proportions of larvae found in the top section of the chamber, particularly at hydrostatic pressures greater than 2.0 m head of water, indicating continued positive phototaxis. The maximum response was observed at 3.0 m hydrostatic pressure. Although the larval distributions observed with an applied light flux of 1500 lux intensity were similar to those found with 1000 lux, the proportions of larvae recorded in the top section were reduced, particularly at hydrostatic pressures less than 2.5 m head of water, suggesting attenuation of the positive phototactic response. The maximum response was observed at 2.5 m hydrostatic pressure.

The variation in the proportion of the population of *S. clava* larvae found in the top section of the vertical behaviour chamber is presented as a three-dimensional surface graph in

Figure 43. Larvae hatched at depth in low light conditions, or the absence of light, will tend to rise in the water column as a result of negative geotaxis; in so doing they will be exposed to increased light intensity and, at a light intensity of about 250 lux, a proportion of the population will tend to sink again forming a circulation cell on the "side of the valley" in Figure 43. Those larvae that pass through this zone continue to rise and are exposed to increasingly higher light intensities which induces positive phototaxis in a positive feed-back system. However, the magnitude of the phototactic response declines as hydrostatic pressure is reduced so a proportion of the larvae will begin to sink as they near the surface. But as they sink, the larvae encounter conditions of light flux and pressure that elicit an increased positive phototactic response and the larvae rise again. This circulation cell keeps a large proportion of the population of *S. clava* larvae in the vicinity of the surface. The valley in the "landscape of distribution" will act as a barrier separating the two circulation cells, which suggests that there should be two populations of larvae, and therefore adults, one near the surface and the other at a depth at which light intensities are below 250 lux.

The "landscape of distribution" appears to be a novel approach to explaining larval response to physical cues. It is an empirical model inspired by the "epigenetic landscape" proposed by Waddington (1957) to explain cell and tissue development. I have attempted to translate the results of a limited number of static experiments into a dynamic qualitative model of larval behaviour for each of the ascidian species studied. Extrapolation from spot observations to a dynamic continuum is always problematic, so the interpretation must be considered tentative.

FIGURE 43 THE PROPORTION OF *S. CLAVA* LARVAE IN THE TOP SECTION OF THE VERTICAL BEHAVIOUR CHAMBER UNDER THE RANGE OF LIGHT INTENSITIES AND HYDROSTATIC PRESSURES EMPLOYED THROUGHOUT THIS STUDY



CHAPTER 13 LARVAL RESPONSES TO LIGHT AND HYDROSTATIC PRESSURE IN COMBINATION

13.1 Introduction

When movement was restricted to the horizontal plane, mature larvae of *Ciona intestinalis* and *Styela clava* exhibited negative phototaxis (Chapter 9). When movement was permitted in the vertical plane, attempts to determine the response to light flux were confounded by gravity and the response could only be deduced (Chapter 10). Nevertheless, with light flux in the vertical plane a small proportion of the *C. intestinalis* larval population appeared to be positively phototactic and a small proportion appeared to be negatively phototactic; *S. clava* and *Ascidiella aspersa* larval populations exhibited weak negative and positive phototaxis respectively. When a range of hydrostatic pressures was applied to the chamber with light flux in the vertical plane, little effect was observed on the distributions of *C. intestinalis* and *A. aspersa* larvae (Chapter 12). However, the weak negative phototaxis exhibited by the *S. clava* larvae changed to positive phototaxis in a large proportion of the population at hydrostatic pressures greater than 1.5 m head of water and light fluxes greater than 1000 lux; the maximum effect was observed with an applied hydrostatic pressure of 2.5 m head of water.

The final combination of behavioural cues to be applied to the larvae is that of light with hydrostatic pressure; this combination should resolve the apparent change in direction of phototaxis of *S. clava*. As time was limited, and the previous experiments indicated that the larvae of *S. clava* were most likely to show a response to light combined with hydrostatic pressure, the experiments were restricted to the larvae of this species.

The horizontal behaviour chamber (see section 5.6) was used in these experiments. For the dark experiments, light was excluded by covering the perspex end-plate with black polythene; for the light experiments, the tube was aligned with the light source by viewing through the bottom hole of the end plate. The top end-plate hole and the bottom tube-holes were then sealed with rubber bungs. A polythene tube (20 mm i.d., 4 m long) was connected via a ¾ inch ball valve and a short length of 6 mm i.d. polythene tube to a short length of glass tube which passed through a rubber bung. The pierced bung was inserted into the bottom end-plate hole. The larger bore polythene tube was filled with filtered (10 μm) aerated sea water and the distal end raised to produce a hydrostatic head of 2.5 m (the hydrostatic pressure that produced maximum effect in Chapter 12).

Filtered (10 μm) aerated sea water (approximately 1900 ml) was poured into the horizontal behaviour chamber via the middle port and allowed to stand for a few minutes to become quiescent. An aliquot (approximately 100 ml) of water and larvae was decanted slowly from the hatching beaker into the chamber through the middle entry port, using a glass funnel with the chamfered edge parallel to the side of the tube so as to avoid directing the momentum of the larvae along the axis of the tube. Rubber bungs were then inserted into the stubs of the entry ports to exclude light. The ball valve was opened very slowly to impose the hydrostatic pressure on the contents of the chamber. The water level in the polythene tube fell as water was transferred to the horizontal chamber, so the tube was topped-up with filtered sea water to re-establish the 2.5 m hydrostatic head and the level monitored for a few minutes to ensure that there were no leaks in the system. The chamber was left for one hour at constant temperature. Natural light was used in the experiments,

which were carried out during periods of near constant light intensity. The light level was checked every few minutes during the experiments (digital luxmeter, Cat No. 610-815, RS Components Ltd) and shading adjusted as necessary, without casting shadows on the perspex window. The experiment was aborted if the light level varied more than ±10% from the target intensity.

After an hour, the ball valve was closed and the tube was rotated to isolate the segments of liquid. The (now) top bungs were removed to reduce the pressure in the chamber to atmospheric pressure and the segments of water drained into labelled sample pots. The segments of tube were rinsed (10 μm filtered sea water) through the top holes, and the samples preserved (section 5.2) for later examination.

A new set of control experiments was carried out as this configuration of apparatus was likely to produce a pressure surge from one end of the behaviour chamber. The perspex end-plate was covered with black polythene and preserved, stained *S. clava* larvae (well rinsed) from recent experiments were introduced into the tube. The hydrostatic pressure was applied and the experiment left for an hour. The ball valve was then closed and the tube rotated to isolate the segments of liquid which were drained into labelled sample pots, stained and preserved as before. This control was designed to determine the distribution of the larvae resulting from the ingress of water from the hydrostatic head, together with any passive movement due to convection currents, vibration of apparatus, etc..

Only mature larvae were used in these experiments. The larvae were maintained under low light intensity conditions prior to experimentation (see section 5.1.4) so as to reduce the risk of habituation to the intensities employed in the experiments.

The distributions of the dead *S. clava* larvae in the control experiments with 2.5 m applied hydrostatic pressure are presented in Table 144. The mean distribution of dead larvae observed in the control experiments with no applied hydrostatic pressure (Table 13, Chapter 5) are included for comparison. The introduction of hydrostatic pressure produced significantly different ($p<0.01$, *G*-test) distributions with significantly greater ($p<0.05$, *t*-test) mean percentages of dead larvae in the end sections (Table 151).

Table 144 Distribution (and %) of dead *S. clava* larvae (control) in the horizontal behaviour chamber with 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, no pressure (Table 13)	34 (1.9)	565 (31.3)	906 (50.2)	259 (14.4)	40 (2.2)	1804
Expt. 1	159 (10.2)	503 (32.2)	667 (42.7)	169 (10.8)	64 (4.1)	1562
Expt. 2	97 (8.6)	260 (23.1)	396 (35.1)	303 (26.9)	71 (6.3)	1127
Expt. 3	234 (10.3)	642 (28.2)	763 (33.5)	441 (19.4)	196 (8.6)	2276
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655

The distributions of *S clava* larvae in the horizontal behaviour chamber after exposure to 2.5 m applied hydrostatic pressure in the absence of light (Table 145) were significantly different ($p<0.01$, *G*-test) to the control distributions and the distributions observed in the absence of applied hydrostatic pressure and light (Table 41). The significances of the differences in mean percentage of larvae in the end sections of the chamber after exposure

to 2.5 m applied hydrostatic pressure in the absence of light are summarized in Table 150. The mean percentages of larvae found in the end sections of the chamber with 2.5 m hydrostatic pressure were significantly different ($p<0.05$, *t*-test) to those found with no applied hydrostatic pressure (Table 151).

Table 145 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with no light and 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655
Expt. 1	38 (10.7)	73 (20.6)	101 (28.5)	76 (21.5)	66 (18.6)	354
Expt. 2	71 (15.6)	106 (23.3)	110 (24.2)	87 (19.1)	81 (17.8)	455
Expt. 3	182 (19.6)	215 (23.2)	238 (25.6)	161 (17.3)	132 (14.2)	928
Mean no pressure (Table 41)	33 (4.4)	250 (28.5)	438 (49.9)	117 (13.4)	33 (3.8)	876

The distributions of *S clava* larvae in the horizontal behaviour chamber after exposure to a light flux of 250 lux and 2.5 m applied hydrostatic pressure (Table 146) were significantly different ($p<0.01$, *G*-test) to the control distributions and the distributions observed in the absence of applied hydrostatic pressure (Table 57). The significances of the differences in mean percentages of larvae in the end sections of the chamber after exposure to 2.5 m applied hydrostatic pressure and light flux of 250 lux are summarised in Table 150. The mean percentages of larvae found in the end sections of the chamber with 2.5 m hydrostatic pressure were significantly different ($p<0.05$, *t*-test) to those found with no applied hydrostatic pressure (Table 151).

Table 146 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 250 lux light intensity and 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655
Expt. 1	37 (22.7)	46 (28.2)	33 (20.2)	23 (14.1)	24 (14.7)	163
Expt. 2	105 (15.3)	159 (23.1)	184 (26.7)	118 (17.2)	122 (17.7)	688
Expt. 3	72 (13.6)	127 (24.1)	134 (25.4)	101 (19.1)	94 (17.8)	528
Mean no pressure (Table 57)	14 (3.2)	113 (26.2)	204 (47.2)	59 (13.6)	42 (9.7)	432

The distributions of *S clava* larvae in the horizontal behaviour chamber after exposure to a light flux of 500 lux and 2.5 m applied hydrostatic pressure (Table 147) were significantly different ($p<0.01$, *G*-test) to the control distributions and the distributions observed in the absence of applied hydrostatic pressure (Table 58).

Table 147 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 500 lux light intensity and 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655
Expt. 1	874 (30.9)	668 (23.6)	582 (20.6)	509 (18.0)	197 (7.0)	2830
Expt. 2	223 (36.4)	164 (26.8)	149 (24.3)	69 (11.3)	8 (1.3)	613
Expt. 3	391 (40.1)	216 (22.2)	191 (19.6)	141 (14.5)	35 (3.6)	974
Mean no pressure (Table 58)	30 (4.7)	124 (19.7)	255 (40.5)	100 (15.9)	121 (19.3)	630

The significances of the differences in mean percentage of larvae in the end sections of the chamber after exposure to 2.5 m applied hydrostatic pressure light at 500 lux are summarised in Table 150. The mean percentage of larvae found in the light end section of the chamber with 2.5 m hydrostatic pressure was significantly different ($p<0.001$, *t*-test) to that found with no applied hydrostatic pressure (Table 151).

The distributions of *S clava* larvae in the horizontal behaviour chamber after exposure to a light flux of 1000 lux and 2.5 m applied hydrostatic pressure (Table 148) were significantly different ($p<0.01$, *G*-test) to the control distributions and the distributions observed in the absence of applied hydrostatic pressure (Table 59). The significances of the differences in mean percentage of larvae in the end sections of the chamber after exposure to 2.5 m applied hydrostatic pressure light at 1000 lux are summarised in Table 150. The mean percentage of larvae found in the light end section of the chamber with 2.5 m hydrostatic pressure was significantly different ($p<0.001$, *t*-test) to that found with no applied hydrostatic pressure (Table 151).

Table 148 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 1000 lux light intensity and 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655
Expt. 1	191 (42.4)	116 (25.8)	89 (19.8)	39 (8.7)	15 (3.3)	450
Expt. 2	404 (45.1)	305 (34.0)	107 (11.9)	48 (5.4)	32 (3.6)	896
Expt. 3	361 (45.4)	274 (34.5)	55 (6.9)	55 (6.9)	50 (6.3)	795
Mean no pressure (Table 59)	25 (4.9)	95 (18.5)	168 (32.7)	127 (24.7)	99 (19.2)	513

The distributions of *S clava* larvae in the horizontal behaviour chamber after exposure to a light flux of 1500 lux and 2.5 m applied hydrostatic pressure (Table 149) were significantly different ($p<0.01$, *G*-test) to the control distributions and the distributions observed in the absence of applied hydrostatic pressure (Table 60). The significances of the differences in mean percentage of larvae in the end sections of the chamber after exposure to 2.5 m applied hydrostatic pressure light at 1500 lux are summarised in Table 150.

Table 149 Distribution (and %) of *S. clava* larvae in the horizontal behaviour chamber with 1500 lux light intensity and 2.5 m applied hydrostatic pressure

	Section A (light)	Section B	Section C	Section D	Section E	Number of larvae
Mean dead control, + pressure	163 (9.9)	468 (28.3)	609 (36.8)	304 (18.4)	110 (6.7)	1655
Expt. 1	456 (46.9)	354 (36.4)	126 (12.9)	31 (3.2)	6 (0.6)	973
Expt. 2	109 (52.4)	33 (15.9)	29 (13.9)	18 (8.7)	19 (9.1)	208
Expt. 3	657 (45.1)	438 (30.1)	272 (18.7)	64 (4.4)	26 (1.8)	1457
Mean no pressure (Table 60)	21 (5.3)	45 (11.4)	159 (39.9)	84 (21.1)	88 (22.2)	397

Table 150 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in section A (light end) and section E (dark end) of the horizontal behaviour chamber with 2.5 m hydrostatic pressure and a variety of light fluxes

(Left-hand side of table (clear) = Section A; right-hand side of table (shaded) = Section E)

Light flux (lux)	Dead	0	250	500	1000	1500
Dead	-	**	*	ns	ns	ns
0	ns	-	ns	*	**	ns
250	ns	ns	-	*	**	ns
500	**	**	**	-	ns	ns
1000	***	*	*	ns	-	ns
1500	***	**	**	*	ns	-

ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

The mean percentage of larvae found in the light end section of the chamber with 2.5 m hydrostatic pressure was significantly different ($p<0.001$, *t*-test) to that found with no applied hydrostatic pressure (Table 151).

Table 151 Significances of differences in mean percentages (*t*-test) of *S. clava* larvae in the light and dark ends of the horizontal behaviour chamber with 0 m and 2.5 m applied hydrostatic pressures and a variety of light fluxes

Significances of differences, ± pressure	Dead	0 lux	250 lux	500 lux	1000 lux	1500 lux
light end (section A)	**	*	*	***	***	***
dark end (section E)	*	***	*	*	*	*

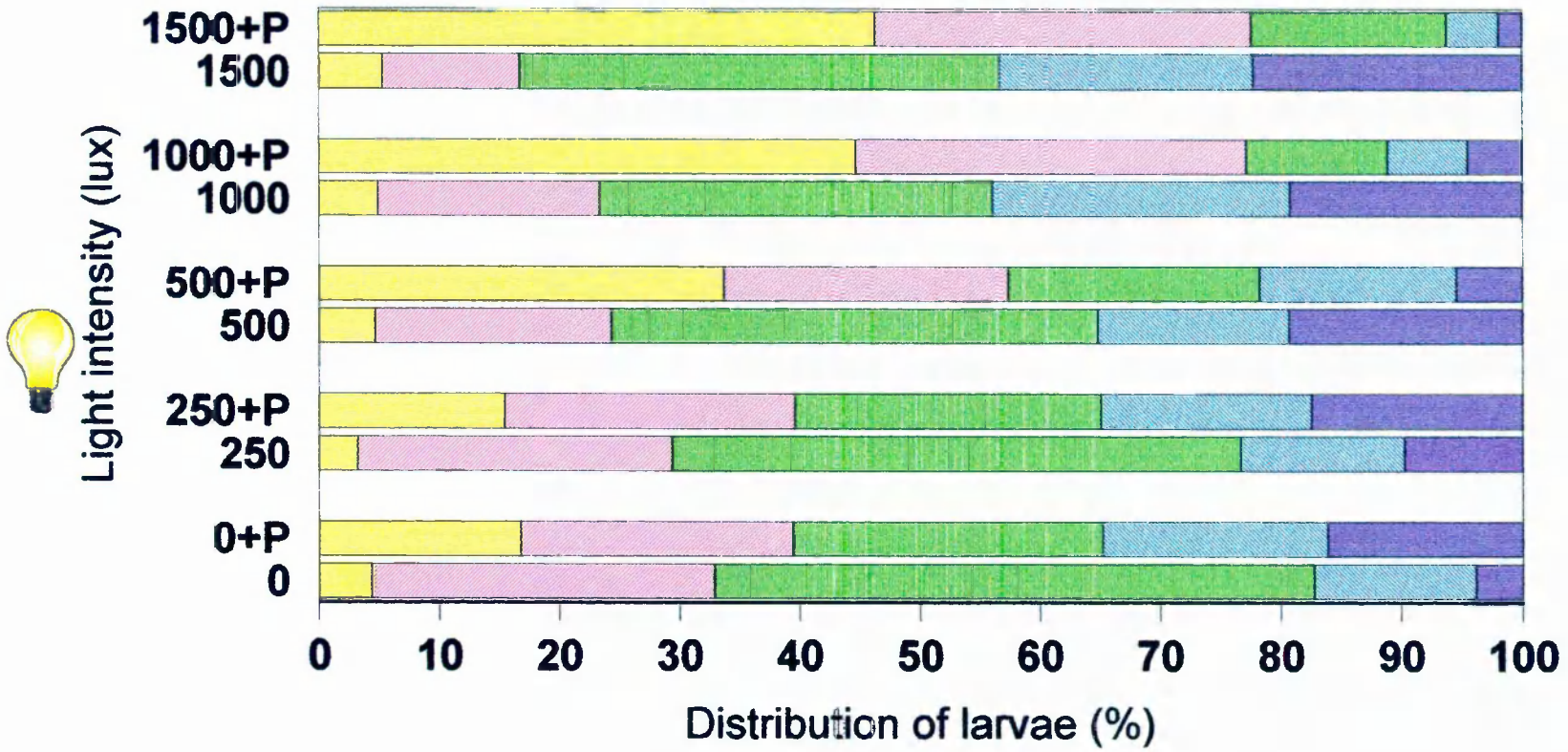
ns = not significant ($p>0.05$); * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

13.4 Discussion

When movement was restricted to the horizontal plane, mature larvae of *Styela clava* exhibited negative phototaxis in the absence of applied hydrostatic pressure. However, when the larvae were exposed to hydrostatic pressure of 2.5 m, they exhibited positive phototaxis at light intensities greater than 250 lux (Figure 44). How can this change in phototactic response of the population of *S. clava* larvae be explained?

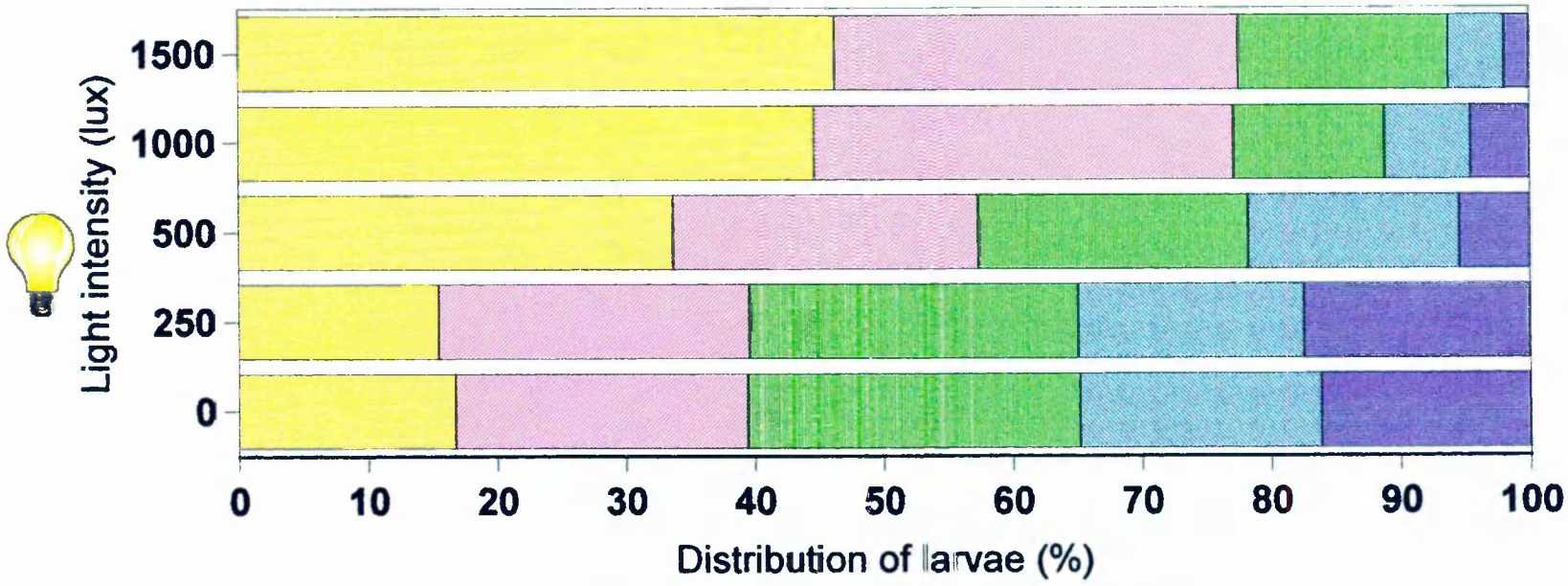
The change in response is unlikely to involve an ontogenetic change in the phototactic response of individual larvae (c.f. *C. intestinalis* larvae in Chapter 8) because only "mature" larvae were used in the experiments. It should be noted that larval maturity was a functional definition in this study (four or more hours old) and was therefore arbitrary; nevertheless, the majority of the larvae used in these experiments were of similar age.

Figure 44 Mean response of mature S. clava larvae to light in the horizontal plane with and without 2.5 m applied hydrostatic pressure



See Figure 12 (page 95) for convention used in Figure 44.

Figure 45 Mean response of mature S. clava larvae to light in the horizontal plane with 2.5 m applied hydrostatic pressure



See Figure 12 (page 95) for convention used in Figure 45.

The proportion of the population moving towards the light source generally increased with applied light intensity (Figure 45), suggesting that the response threshold of an increasing proportion of the population is exceeded as light intensity is increased. But light cannot be responsible for the change in phototactic response since this cue was the same in the experiments with and without pressure. The change in the population response must result from the change in hydrostatic pressure, i.e. a barokinesis effect is involved. Comparison of the distribution of larvae exposed, in the absence of light, to hydrostatic pressure of 2.5 m with that of larvae exposed to no applied hydrostatic pressure indicates that motion has been initiated by the introduction of hydrostatic pressure, so the response results from high barokinesis (Fraenkel and Gunn, 1961). The motion is random in the absence of light, but is manifested as positive phototaxis when a light flux of 500-1000 lux is applied. Photokinesis can be excluded as it would not produce increased larval movement in the absence of light.

It would appear that one portion of the population is weakly negatively phototactic and another portion of the population is positively phototactic. In the absence of hydrostatic pressure the positively phototactic portion are inactive and weak negative phototaxis is exhibited by the other portion of the population. But when hydrostatic pressure is applied the positively phototactic portion of the population become activated and swamp the response of the negatively phototactic portion of the population. Thus the population exhibits positive phototaxis when light and hydrostatic pressure are applied.

The proportion of the population exhibiting positive phototaxis at light intensities of between 1000 and 1500 lux exceeds 30%, thus this response can be considered characteristic. Since only mature larvae will be competent, these results will be directly relevant to larval selection of settlement sites.

CHAPTER 14 FIELD EXPERIMENTS

14.1 Introduction

Three types of field experiment were carried out. In the first, a continuous band of settlement substratum was deployed in the water column to verify the presence of competent larvae at the depths predicted by the fouling panel and laboratory experiments. The second was a transfer experiment in which panels fouled at different depths were exchanged to distinguish between the effects of depth on settlement and on juvenile mortality. In the third set of field experiments an attempt was made to determine the vertical distribution of larvae in the water column.

14.2 Recruitment on continuous substratum

The discrete nature of fouling panels (Chapter 4) provides an intermittent view of the recruitment of fouling organisms throughout the water column; settlement is governed by the presence of substratum. To verify the occurrence of the pre-settlement zonation predicted by the laboratory experiments, it is necessary to provide a continuous band of substrate stretching through the water column. The rope supporting the settlement panels in the earlier experiments partially fulfilled this role, but the presence of the panels made comprehensive census difficult as they obscured large sections of rope and may have influenced the settlement of larvae by affecting the local hydrography. Therefore the basic recruitment experiments were repeated using only the rope as a continuous band of settlement substratum.

14.2.1 Methods

Three lengths of rope (3 m), which had been aged in sand filtered water for six months, were suspended from the oil-boom on April 16, 1993. Chains were used to weigh down the ropes and the rope lengths were adjusted so that the chains just cleared the bottom at spring tide low water (maximum depth was about 2.5 m). One rope was removed and examined after twelve weeks, a second after six months and the third after a year. The ropes were censused in approximately 10 cm sections.

14.2.2 Results

After twelve weeks there was intense recruitment of *A. aspersa* at about 2 m depth (Plate 43); most *C. intestinalis* present were found at this depth. A few specimens also occurred between 0.5 and 1.5 m (Figure 47), but *S. clava* was not recorded as the rope was removed before its breeding season. The numbers of *A. aspersa* and *C. intestinalis* were considerably reduced after six months (Plate 44) compared with twelve weeks. *A. aspersa* was almost completely restricted to a depth of approximately 2 m (Plate 45), and *C. intestinalis* to the bottom of the rope and chain (Plate 46) *S. clava* settled just below the surface, at 0.5 m depth (Plate 47) and at about 2 m depth (Figure 48).

After twelve months the bottom of the rope was laden with ascidians (Plate 48). The main recruitment of *A. aspersa* and *C. intestinalis* occurred between 1.8 m and 2.2 m depth, and that of *S. clava* between 0.2 m and 0.7 m depth with some recruitment around 2 m depth. *C. intestinalis* occurred sporadically below 1 m depth (Figure 49).

Plate 43 **Ascidian recruitment on rope after 12 weeks exposure (1993)**

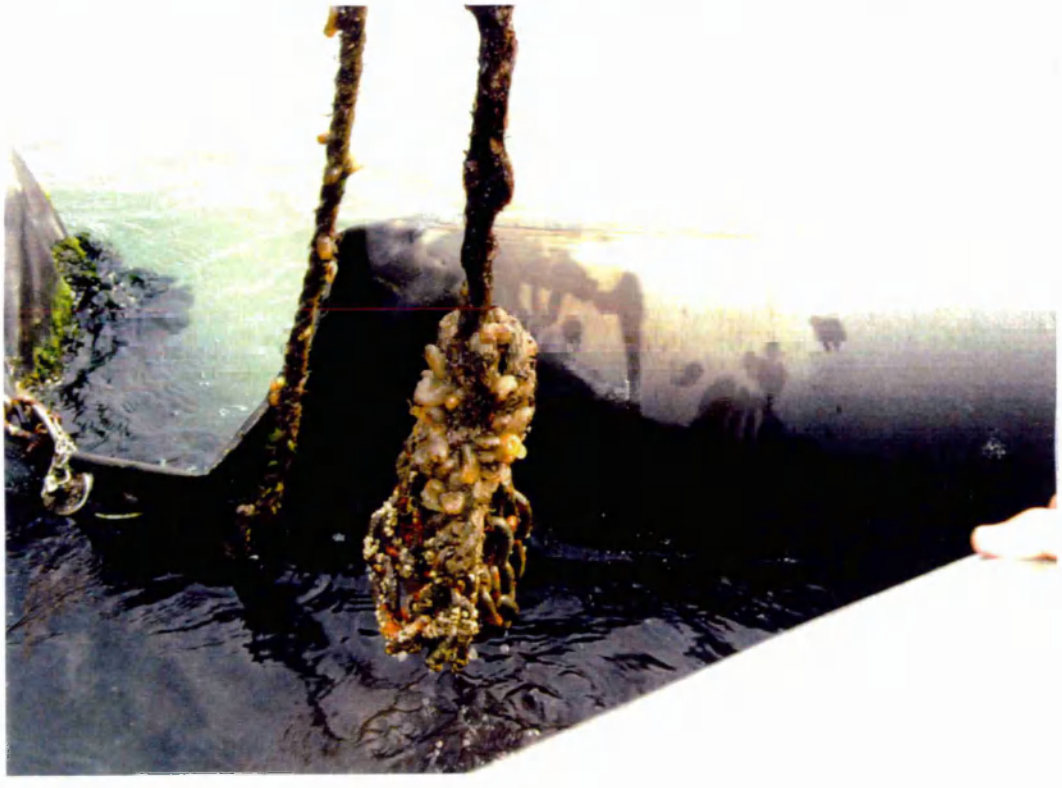


Plate 44 **Ascidian recruitment on rope after 6 months exposure (1993)**

Plate 45 **Detail of *Ascidiella aspersa* on the rope at 2 m depth after 6 months**

Plate 46 ***Ciona intestinalis* attached to the bottom of the rope after 6 months**

Plate 47 ***Styela clava*** **attached to the top end of the rope after 6 months**

Plate 48 **Ascidian recruitment on rope after 12 months exposure (1993/4)**

Figure 46 Ascidian recruitment on rope at constant depth after 12 weeks (1993)

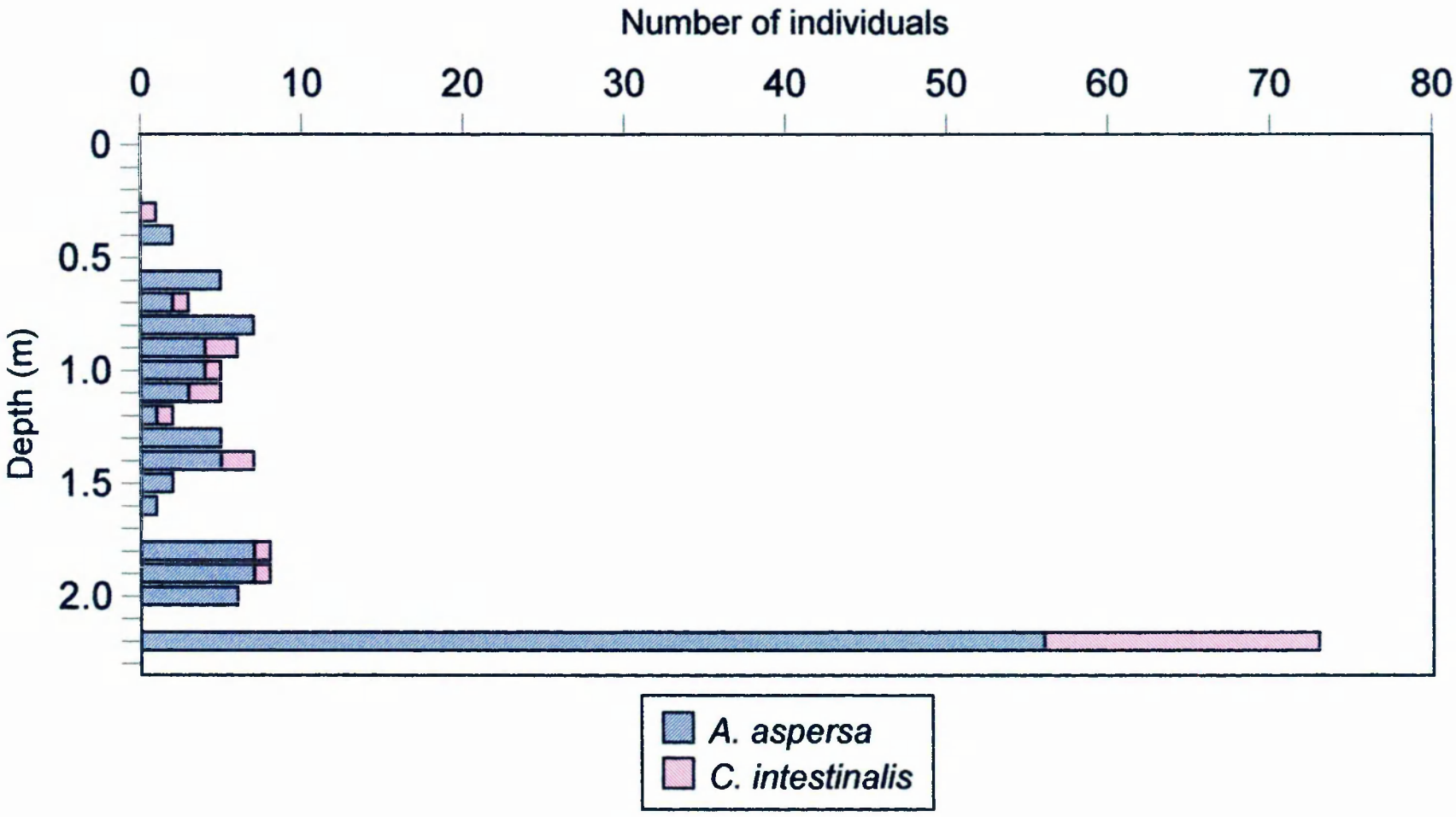


Figure 47 Ascidian recruitment on rope at constant depth after 6 months (1993)

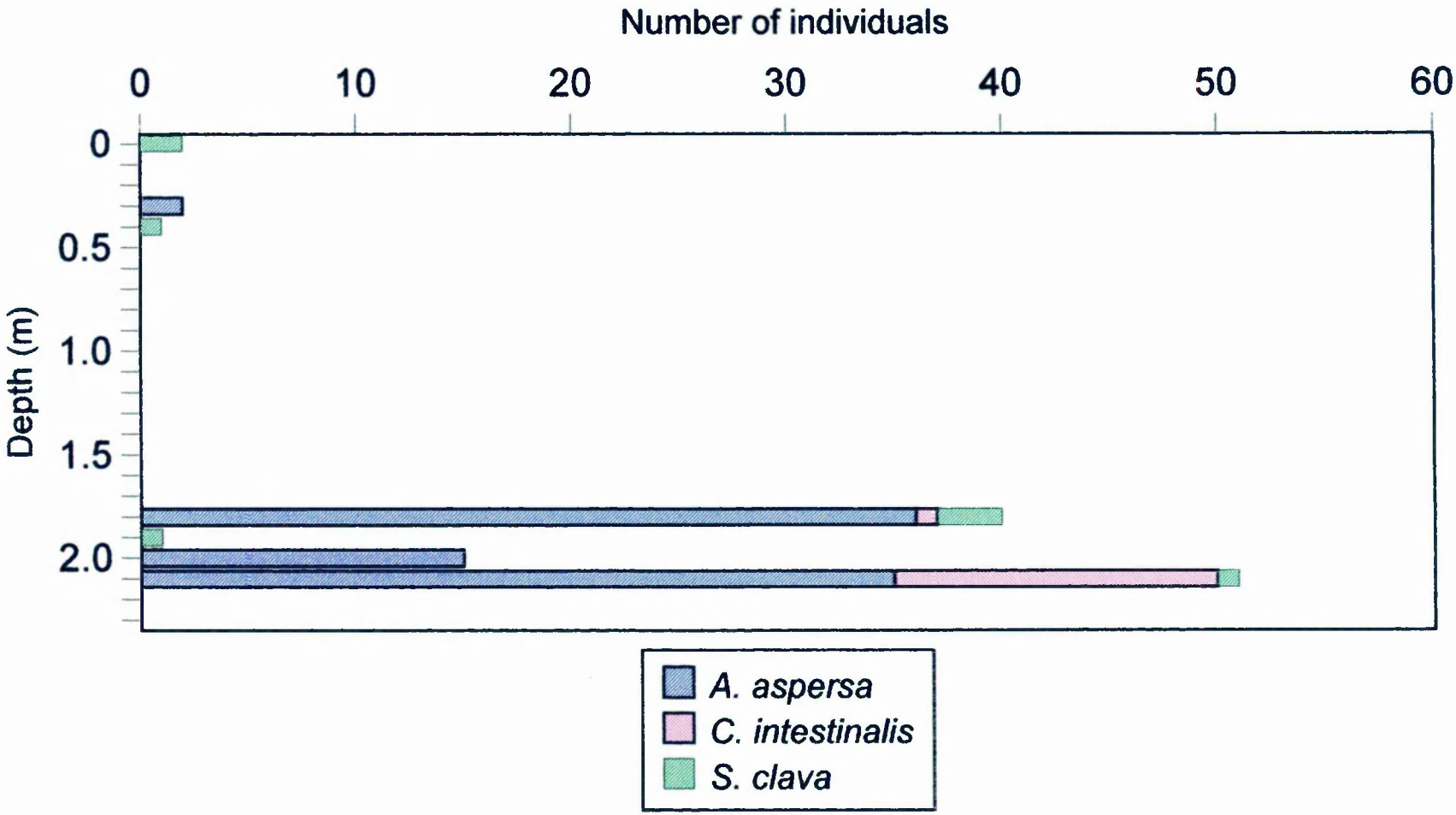
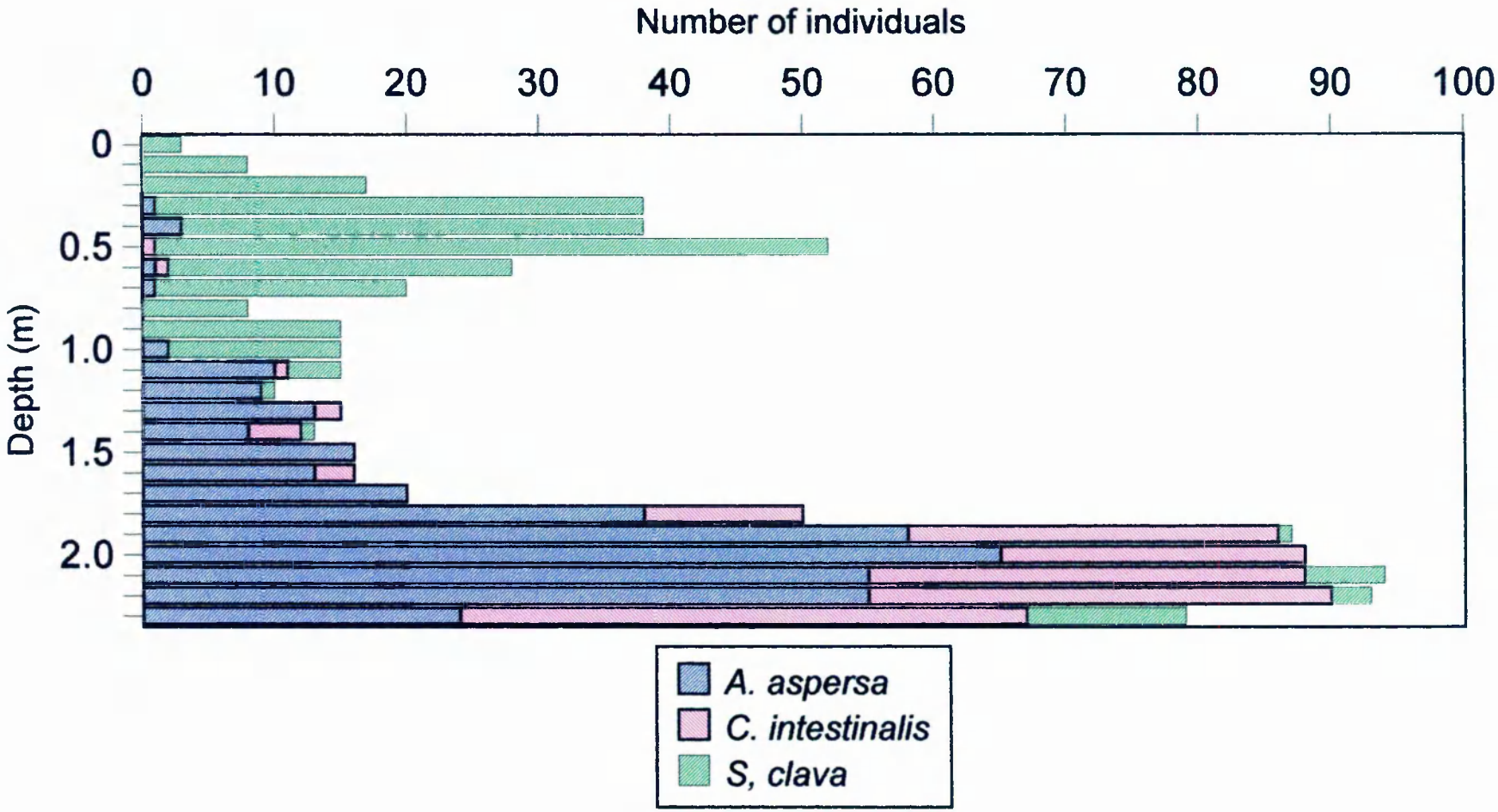


Figure 48 Ascidian recruitment on rope at constant depth after one year (1993-4)



14.2.3 Discussion

The results indicate that the zonation of recruitment observed on the fouling panels is not an artefact produced by the presence of the panels, but also occurs when a continuous band of substratum is suspended in the water column. These observations support the larval zonation hypothesis. Comparison of recruitment after 12 weeks and one year suggests that there may be competitive displacement of *A. aspersa* and *C. intestinalis* by *S. clava* between 0 m and 1 m depth.

14.3 Panel exchange experiment

An attempt was made to identify the contribution of juvenile mortality to the recruitment distribution observed with depth by moving recently colonized panels from previously identified zones of high recruitment to zones of low recruitment and *vice versa*. The exchange panel array was re-exposed for a period before census and the ascidian population recruited on it was compared with that on a control array exposed for the same overall period at the same site.

14.3.1 Methods

Three of the arrays of ropes and panels deployed in 1992 (Section 4.2.3) were reused in this experiment; the panel spacings were the same as used previously. All initial attachments were made with black cable ties threaded through the rope and the holes in the corners of the panels. The ropes with attached panels were suspended from the seaward side of the oil-boom on May 23, 1993.

One array was removed for census on August 15, 1993. At the same time panels 1 (top) and 3, and 2 and 4 (bottom) of another array were exchanged; the panels were reattached with yellow cable ties so as to identify the exchange array. The third (control) array was not disturbed. The two remaining arrays were removed after 21 weeks total exposure, on October 17, 1993. The numbers of each of the ascidian species of interest on each panel were recorded.

14.3.2 Results

After twelve weeks exposure, one side of the surface panel was 80% covered with a polyzoan. (Plate 49), the other side was fouled with red algae. The panel from 1 m depth was fouled with red algae, numerous barnacles and a few small *A. aspersa* and *C. intestinalis* (Plate 50). The density of juvenile *A. aspersa* specimens increased on the panel from 2 m depth (Plate 51). and declined slightly on the panel from 3 m depth (Plate 52). The numbers of *C. intestinalis* specimens on these last two panels were approximately triple and double, respectively, that on the panel from 1 m depth. (Table 152).

Table 152 Distribution of solitary ascidians with depth after 12 weeks (1993)

Depth (m)	*A. aspersa*	*C. intestinalis*	*S. clava*
0	0	0	0
1	12	10	0
2	164	29	0
3	107	21	0

Plate 49 Fouling community on surface panel after 12 weeks (1993)

Plate 50 Fouling community on panel from 1 m depth after 12 weeks (1993)

Plate 51 **Fouling community on panel from 2 m depth after 12 weeks (1993)**

Plate 52 **Fouling community on panel from 3 m depth after 12 weeks (1993)**

After 21 weeks exposure the surface panel of the control set was fouled with red algae, barnacles, moderate numbers of *A. aspersa* and *S. clava*, and a few *C. intestinalis* (Plate 53). The panel that had been exposed at 1 m depth supported a larger population of *A. aspersa* and *C. intestinalis* than the surface panel; the number of *S. clava*, and the percentage cover of red algae and barnacles, were similar to the surface panel (Plate 54). The panel that had been exposed at 2 m depth panel was about 90% covered with *A. aspersa* and *C. intestinalis*, with a few *S. clava* and slightly more red algae than was found on the 1 m depth panel (Plate 55). The panel that had been exposed at 3 m depth panel (Plate 56) supported a slightly smaller population of *A. aspersa* and *C. intestinalis*, but an increased number of *S. clava* (Table 153). The rope connecting the deepest panels was colonised with all three ascidian species.

The surface panel of the exchange array was fouled with a large quantity of red algae, barnacles, *A. aspersa* and small *S. clava*, with some *C. intestinalis* (Plate 57). This panel had been submerged at 2 m depth for the first 12 weeks. The panel from 1 m depth was fouled with less red algae, barnacles and *A. aspersa* than the surface panel, with approximately equal numbers of *C. intestinalis* and small *S. clava* (Plate 58). This panel had been submerged at 3 m depth for the first 12 weeks. The panel from 2 m depth was fouled with a small quantity of red algae, barnacles, *A. aspersa*, small *S. clava* and *C. intestinalis*, but large numbers of serpulid worms (Plate 59). This panel had been exposed at the surface for the first 12 weeks. The panel from 3 m depth had similar populations of *A. aspersa* and *C. intestinalis* to the panel from 2 m depth, but supported a larger number of *S. clava*; it was less fouled with serpulid worms and red algae (Plate 60). This panel had been submerged at 1 m depth for the first 12 weeks.. The numbers of ascidians found on the panels in this experiment are presented in Table 153.

Plate 53 Fouling community on surface control panel after 21 weeks (1993)

Plate 54 Fouling community on control panel from 1 m depth after 21 weeks

Plate 55 **Fouling community on control panel from 2 m depth after 21 weeks**

Plate 56 **Fouling community on control panel from 3 m depth after 21 weeks**

Plate 57 Fouling community on surface exchange panel after 21 weeks (1993)

Plate 58 Fouling community on exchange panel from 1 m depth after 21 weeks

Plate 59 Fouling community on exchange panel from 2 m depth after 21 weeks

Plate 60 Fouling community on exchange panel from 3 m depth after 21 weeks

Table153 Distribution of solitary ascidians with depth after 21 weeks (1993)

Depth (m)	*A. aspersa*		*C. intestinalis*		*S. clava*	
	Control	Exchange	Control	Exchange	Control	Exchange
0	65	493 (init. 2m)	14	49 (init. 2m)	164	123 (init. 2m)
1	250	346 (init. 3m)	58	45 (init. 3m)	127	104 (init. 3m)
2	587	4 (init. 0m)	80	8 (init. 0m)	11	7 (init. 0m)
3	438	12 (init. 1m)	60	6 (init. 1m)	63	38 (init. 1m)

init. = initial exposure depth (m)

14.3.3 Discussion

The exchange panels showed a similar trend in recruitment of *A. aspersa* and *C. intestinalis* to the control panels from the same original exposure depth, i.e. greatest recruitment at 2 m depth, least at the surface. Recruitment on the undisturbed 2 m and 3 m panels after 21 weeks was substantially greater than on the 12 week control panels, but recruitment on the re-sited 2 m and 3 m panels did not show a similar increase. This could be because little settlement occurred after August 15 that year, but the majority of that which did take place was at 1 m depth. (Ascidian densities were probably higher on the twenty-one week panels than on the twelve week panels because recently settled larvae on the latter would have been too small to see and count). Alternatively, there may have been higher mortality of recently settled larvae on plates moved from shallow to deep stations. The specimens of *A. aspersa* and *C. intestinalis* growing on the ropes between 2 m and 3 m depth (Plates 59 and 60) indicate that the lack of ascidians on the relocated plates was unlikely to be due to juvenile mortality, but new recruits may have been more vulnerable.

The numbers of *S. clava* found on the exchange panels are similar to, but smaller than, those observed on the control panels at each depth. This suggests that these larvae were recruited after August 15 in that year. It should be noted that the juvenile *S. clava* were very small and it was possible that some were missed when the plates were censused, so the results may be an underestimate.

Comparison of the results after twelve weeks and twenty-one weeks (control) indicates that the distributions of *A. aspersa* and *C. intestinalis* do not change with increased exposure time. Comparison of the control and exchange panels after twenty one weeks indicates that the distributions of *A. aspersa* and *C. intestinalis* do not change when the exposure situation is changed, suggesting that differential recruitment is not the result of enhanced juvenile mortality at shallow depth.

14.4 Direct sampling of larvae in the water column

14.4.1 Method

A submersible sampler (Plate 61) was constructed by mounting a 95 μm nylon mesh sleeve in a plastic tube which was attached to the suction side of a submersible pump (Coughlan & Fleming, 1978). A plastic ring was attached to the front end of the nylon netting to keep the sleeve open, and a plastic bottle was fitted to the rear end to collect the sample (Plate 62). The current velocity at the sampling orifice (64 mm diameter) was estimated at 2.85 m s^{-1}, more than sufficient to prevent larvae from escaping. The volume sampled was calculated from a calibrated flow meter fitted in the mouth of the pump-sampler.

Plate 61 The submersible pump-sampler

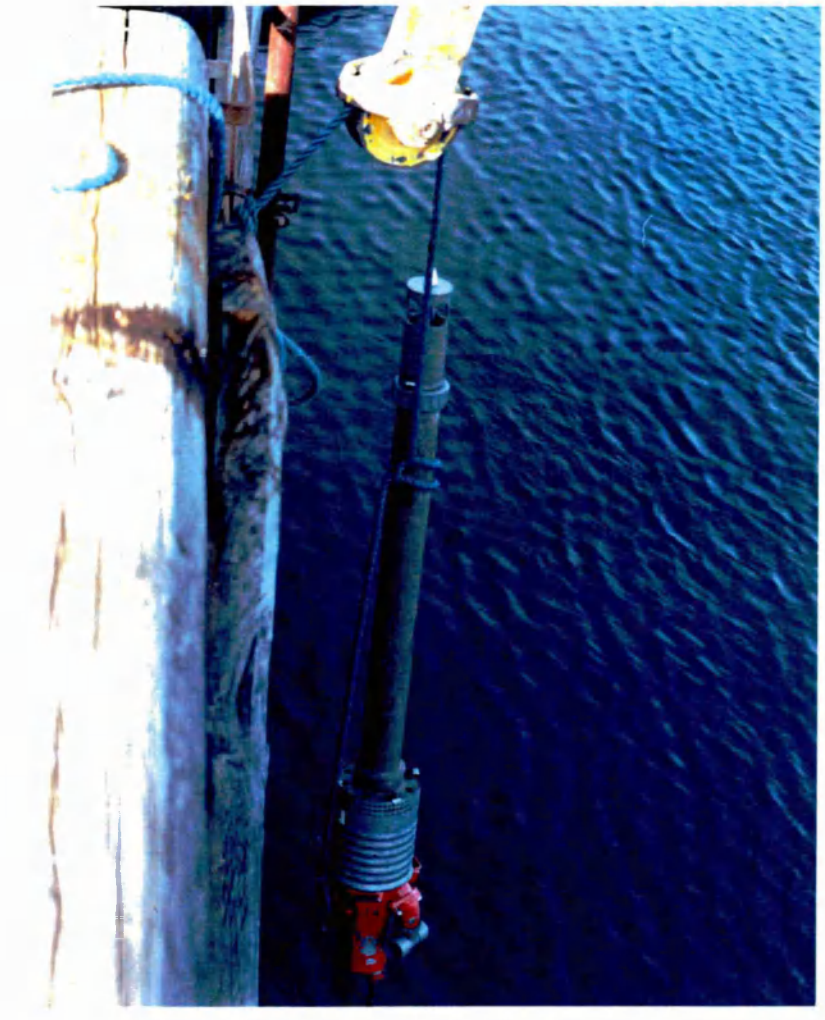
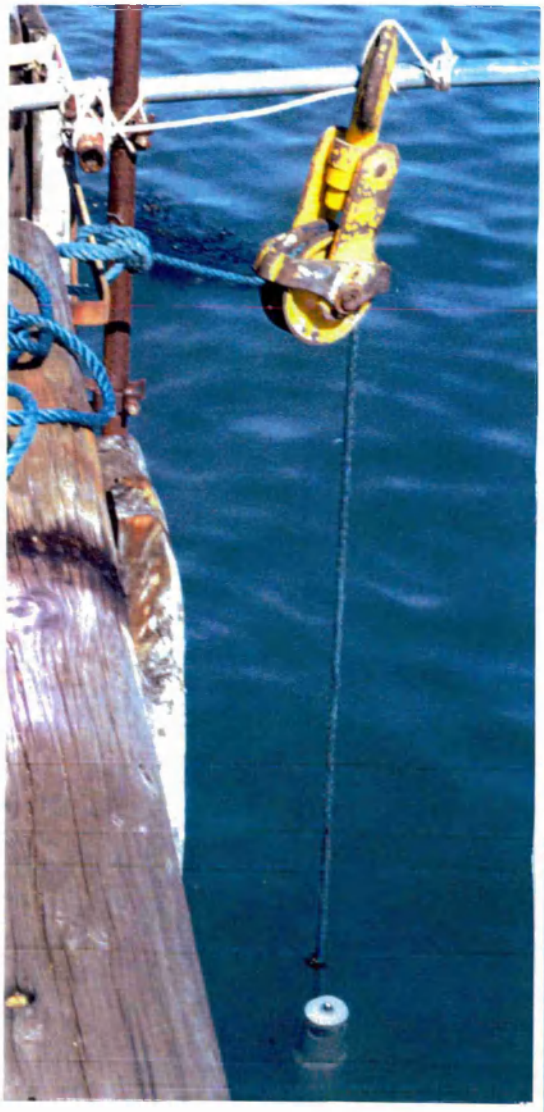


Plate 62 The nylon mesh sleeve with sample collector

Plate 63 The pump-sampler deployed in the intake dock



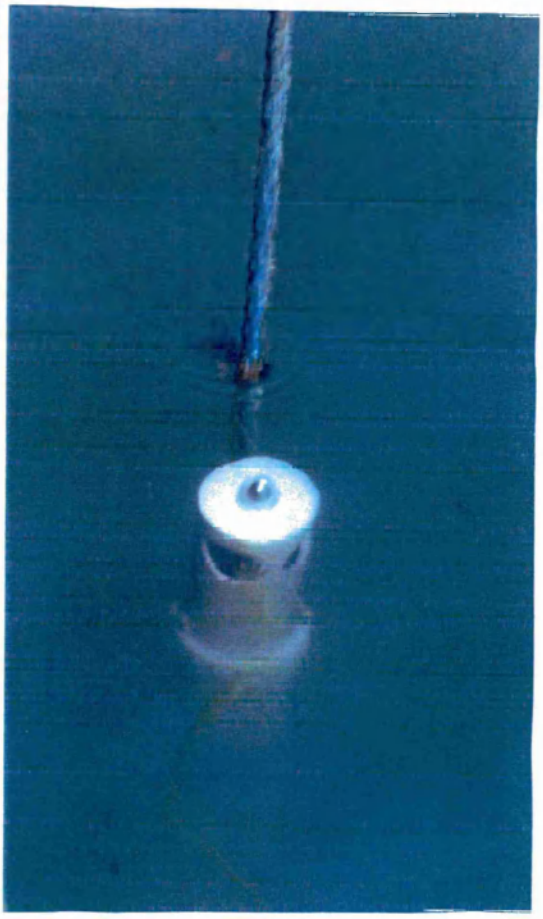


Plate 64 Detail of the pump collecting a surface sample

The flow meter reading was recorded and the pump-sampler was lowered into the inlet dock just land-ward of the oil-boom, approximately 0.5 m from the dock wall, with the intake orifice positioned just below the surface (Plates 63 & 64). The rope supporting the pump was marked at the tying-off point. A timed sample (approximately 20 minutes) was taken at the surface, the sampler recovered and the flow meter reading noted. All material adhering to the inside of the plankton netting sleeve was rinsed down (from the outside) into the sample pot. The sample was transferred, with rinsing, to a labelled jar and preserved with formaldehyde and Rose Bengal for later examination (section 5.2). Sampling was repeated at 0.5 m intervals to the bottom of the inlet by paying out 50 cm increments of the rope supporting the pump.

Sampling was opportunistic, taking place when other experimentation permitted. Although the sampling regime was sporadic, at least two sampling surveys were attempted during the predicted spawning season of each species. Three sampling surveys were carried out in early September 1991 and two in early September 1993, when the larvae of *S. clava* were expected to be in the water column. Two sampling surveys were carried out in June 1994, when the larvae of *C. intestinalis* were expected to be in the water column. One sampling survey was carried out late June and one in early August 1994, when the larvae of *A. aspersa* were expected to be in the water column.

14.4.2 Results

A few *C. intestinalis* larvae were caught at depths between 1 m and 2 m in the first sampling survey carried out in September 1991; the distribution differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in the

vertical distribution of *C. intestinalis* larvae. No *A. aspersa* larvae or *S. clava* larvae were caught in this survey (Table 154).

Table 154 Larvae sampled in water column, 8/9/91 (HW 1130h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1314	10.535	0	0	0
0.5	1409	10.803	0	0	0
1.0	1447	8.443	3	0	0
1.5	1523	10.645	5	0	0
2.0	1555	10.474	11	0	0
2.5	1620	10.292	0	0	0
3.0	1650	10.012	0	0	0

Similar numbers of *C. intestinalis* larvae were caught at similar depths in the second survey; the distribution differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in the vertical distribution of *C. intestinalis* larvae. Two *S. clava* larvae were caught at shallow depth, but no *A. aspersa* larvae were caught in this survey (Table 155).

Table 155 Larvae sampled in water column, 9/9/91 (HW 1210h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1524	5.516	0	0	0
0.5	1546	10.894	0	0	1
1.0	1605	5.832	0	0	1
1.5	1623	5.820	3	0	0
2.0	1645	10.474	4	0	0
2.5	1702	10.292	13	0	0

In the third September 1991 sampling survey, the number of *C. intestinalis* larvae caught declined but the number of *S. clava* larvae caught increased (Table 156). The majority of the *C. intestinalis* larvae were found at depth; the distribution differed significantly ($p<0.05$,

χ^2-test) from random indicating that depth was a significant determinant in larval vertical distribution. The majority of the *S. clava* larvae were found near the surface; larval distribution differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in the vertical distribution of *S. clava* larvae. No *A. aspersa* larvae were caught in this survey.

Table 156 Larvae sampled in water column, 10/9/91 (HW 1250h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1326	7.488	0	0	5
0.5	1405	5.878	0	0	17
1.0	1439	5.698	1	0	3
1.5	1513	6.678	0	0	0
2.0	1548	3.424	0	0	0
2.5	1615	5.866	1	0	0
3.0	1658	6.062	4	0	1

In September 1993, only two *C. intestinalis* larvae were caught in the first sampling survey (Table 157). The majority of the *S. clava* larvae caught were found in samples collected close to the surface; larval distribution differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in the vertical distribution of *S. clava* larvae. No *A. aspersa* larvae were found in any of the samples collected during this sampling survey.

Table 157 Larvae sampled in water column, 7/9/93 (HW 1509h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1135	5.748	0	0	4
0.5	1204	5.788	0	0	11
1.0	1234	5.928	1	0	3
1.5	1301	5.767	0	0	0
2.0	1323	5.344	0	0	0
2.5	1354	5.616	1	0	0

Similar numbers of *C. intestinalis* larvae and *S. clava* larvae to those caught in the first 1993 sampling survey were caught in the second survey, carried out three days later (Table 158). The majority of the *S. clava* larvae caught were again found in samples collected close to the surface; larval distribution differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in the vertical distribution of *S. clava* larvae. No *A. aspersa* larvae were caught in this sampling survey.

Table 158 Larvae sampled in water column, 10/9/93 (HW 1812h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1121	5.816	0	0	1
0.5	1205	5.628	1	0	14
1.0	1240	6.169	0	0	2
1.5	1314	5.381	0	0	0
2.0	1346	6.044	0	0	0
2.5	1415	5.822	3	0	1

In June 1994, no *A. aspersa* larvae or *S. clava* larvae were caught in the first sampling survey (Table 159), and the majority of the *C. intestinalis* larvae caught were found in samples collected at depths greater than 2 m. The distribution of *C. intestinalis* larvae differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in their vertical distribution.

Table 159 Larvae sampled in water column, 22/6/94 (HW 1025h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1055	7.488	0	0	0
0.5	1130	5.878	0	0	0
1.0	1203	5.698	4	0	0
1.5	1230	6.678	3	0	0
2.0	1257	3.424	22	0	0
2.5	1332	5.866	14	0	0

More *C. intestinalis* larvae were caught in the second 1994 sampling survey, carried out four days later (Table 160), the majority occurring at depths greater than 1.5 m. The distribution of *C. intestinalis* larvae differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in their vertical distribution. No *A. aspersa* larvae or *S. clava* larvae were caught in this sampling survey.

Table 160 Larvae sampled in water column, 26/6/94 (HW 1343h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1150	7.488	3	0	0
0.5	1225	5.878	1	0	0
1.0	1253	5.698	6	0	0
1.5	1326	6.678	12	0	0
2.0	1402	3.424	17	0	0
2.5	1436	5.866	21	0	0
3.0	1508	6.062	7	0	0

The majority of *C. intestinalis* larvae caught in the sampling survey carried out in July 1994 (Table 161) were caught at depths greater than 2 m; the distribution of these larvae differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in their vertical distribution. Given the time of year, fewer than expected *A. aspersa* larvae were caught in this survey; all were collected at depths greater than 1.5 m. The distribution of the *A. aspersa* larvae differed significantly ($p<0.05$, χ^2-test) from random indicating that depth was a significant determinant in their vertical distribution. No *S. clava* larvae were caught in this sampling survey.

Table 161 Larvae sampled in water column, 23/7/94 (HW 1159h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1101	6.161	0	0	0
0.5	1129	5.932	7	0	0
1.0	1158	6.119	3	0	0
1.5	1224	6.231	4	1	0
2.0	1256	6.012	10	4	0
2.5	1336	5.984	23	4	0

Fewer *A. aspersa* larvae and *C. intestinalis* larvae were caught in the sampling carried out in August 1994 (Table 162); all larvae were again collected at 1.5 m depth or greater. The distribution of *C. intestinalis* larvae differed significantly ($p<0.01$, χ^2-test) from random indicating that depth was a significant determinant in their vertical distribution. No *S. clava* larvae were caught in this sampling survey.

Table 162 Larvae sampled in water column, 6/8/94 (HW 1103h BST)

Sample depth (m)	Time (h, BST)	Volume (m^3)	No. of *C. intestinalis*	No. of *A. aspersa*	No. of *S. clava*
Surface	1031	6.161	0	0	0
0.5	1102	5.932	0	0	0
1.0	1128	6.119	0	0	0
1.5	1159	6.231	0	1	0
2.0	1234	6.012	1	1	0
2.5	1306	5.984	7	1	0

14.4.3 Discussion

The larval concentrations observed in these experiments seem low, particularly since dense populations of solitary ascidians grew on the nearby dock wall. Low larval numbers could have resulted from net damage; but none of the larvae were severely distorted, all were

identifiable and there was no evidence of larval fragments. The net mesh could have been too large, permitting larval egress; however, this mesh size was used to filter the water from the spawning tanks (section 5.1.3) and recently hatched larvae were often retained with the eggs. The pump-sampler has been shown to collect representative samples (Coughlan & Fleming, 1978) so the low larval concentrations recorded were probably due to the sampling exercises taking place when larvae were not abundant. Diel time of sampling should not influence the numbers of larvae caught as experiments were carried out when the larvae were likely to be present in the water column, based on laboratory hatching times (section 6.3). It is possible that currents local to the sampling point may have influenced the numbers of larvae captured; the sampling site was just downstream of the oil-boom (section 2.2) and the pump-sampler was suspended only 0.5 m from the dock wall.

In some of the sampling experiments, particularly those that took place at mid-tide, there was a problem allocating the few larvae caught to a specific depth zone because the tide rose or fell substantially during the sampling period. Nevertheless, the distributions of the few larvae that were collected support the hypotheses that *C. intestinalis* larvae tend to concentrate below 1.5 m depth in the water column, and *S. clava* larvae tend to concentrate near the surface. In addition, the distributions of the small number of *A. aspersa* larvae collected do not refute the hypotheses that these larvae tend to concentrate below 1.5 m depth in the water column. However, the numbers of larvae caught were too small to draw any firm conclusions.

CHAPTER 15 GENERAL DISCUSSION

Three species of solitary ascidians coexist in abundance on submerged substrata in Southampton Water, in apparent contradiction of the premise that two or more species existing in the same habitat and utilising the same limited resources would be in competition and only the competitively superior individuals would get enough of the resources to survive (Nicholson, 1954). This premise is derived from the competitive exclusion principle (Gause, 1934) which states that, in a stable environment, two species can only coexist if a niche difference occurs between them. Hardin (1960) extended the principle by proposing that if no niche difference exists then the superior competitor will eliminate the inferior one from that habitat. Thus the coexistence of the ascidian species requires explanation, particularly since one is alien to this location. Initial observation suggests that there is environmental stability, within an annual cycle, and the three species are likely to be competitors unless they exploit different niches. They have similar life cycles (Berrill, 1950; Holmes, 1968), general distributions within Southampton Water (Holmes, 1968; 1971; Collins & Mallinson, 1987), feeding methods and diets (Jørgensen, 1949; Jørgensen & Goldberg, 1953; Holmes, 1968; Randløv & Riisgård, 1979); so is distribution governed by competition? General observation during this study suggested that none of the species suffered heavy predation, although Gulliksen & Skjæveland (1973) and Svane (1983) identified the starfish *Asterias rubens* L. as a major predator of *Ciona intestinalis* in Norwegian and Swedish waters respectively; none of the species carried a heavy parasite load or appeared to be disease controlled, so these factors are not likely to be critical.

The generalization that no two species of organisms can occupy the same ecological niche at the same time, termed Gause's law (Lack, 1949), has generated controversy. Ross (1957)

found different species of sycamore leafhopper occupying the same ecological niche at the same time, with no apparent interspecific competition. To explain this result, he proposed that the ecological niche or habitat which served as the range of a species was not usually uniform, but was "a kaleidoscope of local and annual variations around certain ecological means"; furthermore, as species were always slightly different physiologically, any two species would have slightly different ecological optima permitting a niche to be occupied and used by more than one species at the same time. He therefore proposed that, when interspecific competition existed, a species would survive only if, under conditions of its own optimum, it could develop larger populations than its companion species, but in the absence of interspecific competition there would be no predictable limit to the number of species that could occupy the same niche. In recent years there has been much discussion on the relevance of Gause's theory to ecology (see, for example, Strong *et al.*, 1984; Shorrocks *et al.*, 1984; den Boer, 1986). The habitat occupied by the three ascidian species studied is obviously not uniform, but it is difficult to see how their distribution can be explained in terms of niche differences when most mature populations contain some individuals of each species. So is interspecific competition operating?

Interspecific competition is usually asymmetric, with one species being affected to a much greater extent than the other (see, for example, Jackson, 1979; Lawton and Hassell, 1981; Grace and Wetzel, 1981). To establish the occurrence of interspecific competition it is necessary to demonstrate that the potential competitors share a common resource. If interspecific competition for food or space were the chief factor governing density of populations, then theoretically the prerequisite for the survival of any one of the coexisting species would be its ability to develop larger populations than the other species in some portion of the ecologically heterogeneous niche. The coexisting complex would therefore be

composed of species each with a different optimum (*sensu* Ross, 1957) and each out-competing all the others under conditions of its own optimum. Is this the situation for populations of solitary ascidians? In the mixed ascidian populations examined in Southampton Water there was usually one species that was dominant in the extreme.

The maximum competition experienced by the species occurring in any particular situation is determined by the similarity of the realised niches in that situation, and the proximity of the populations to the carrying capacity of the environment (Begon *et al.*, 1990). Competition between solitary ascidian species, as for most sessile species, is most acute during the larval settlement season. For such sessile species, competition is for settlement space, and food supply is unlikely to present as great a problem. Indeed, in the breeding season, recruitment of solitary ascidians readily occurs when new substrate is introduced into the water column indicating that settlement space, not food, is the limiting factor in recruitment. Having established that competition for space occurs within and between species, the mechanism of coexistence requires explanation.

The first question that must be addressed is "Is competition the major organising force in the community structure of these three ascidians?" If it is not the major force, then "How has the strength of interspecific competition been reduced so as to permit coexistence?" and "What survival strategy permits these solitary ascidian species to coexist in such abundance?". Of special interest is the relationship of the introduced species to the indigenous ascidians. These questions will now be discussed in the light of the experiments carried out in this study and of published information.

The observation that in harbours where *Styela clava* does not occur, neither *Ciona intestinalis* nor *Ascidiella aspersa* occupies the vacant habitat, and the occurrence of mixed populations of the three species in Southampton Water, suggest that competition *per se* is not the major factor determining community structure. To survive in the short term animals must, of course, be adapted to the particular environments in which they live; in the case of the immigrant species *S. clava*, the temperature regimes of southern English waters are similar to those of its original range (Wallace, 1961) and are suitable for it to breed successfully. Longer term survival depends on other population properties, characterised as strategies, which involve flexibility of response to environmental factors - described by Levins, (1968) as "adaptations to the pattern of the environment in space and time". Many of the strategies suggested for the avoidance of competition were described in the introduction to this thesis. Although feeding strategy can reduce potential competition with other filter feeders, it is unlikely to play a role in this case because the food gathering apparatus of each of the three ascidian species is relatively large in relation to the size of food particles consumed (Jørgensen and Goldberg,, 1953; Jørgensen, 1955; Holmes, 1968; Randløv & Riisgård, 1979), so they have the ability to feed on a wide range of food particle sizes in a relatively unspecialised manner. Avoidance strategies, such as niche differentiation (Connell, 1980; Begon *et al.*, 1990) can permit coexistence, and a similar mechanism would appear to be involved in the successful coexistence of these ascidian species. But adult ascidians are sedentary animals; therefore any niche differentiation that occurs must be the result of larval selection of settlement site and/or selective juvenile mortality.

The period of most intense competition will occur when there are most competitors present, i.e. the period when larvae are settling. Reproducing at different times of the year will ameliorate interspecific competition between larvae. This strategy, which tends to reduce

competition for settlement sites and maximises species recruitment, may partially explain the successful coexistence of *A. aspersa* and *S. clava*, which breed at the beginning and end of the summer period respectively. Intraspecific competition will not, of course, be affected by this segregation of breeding seasons. *C. intestinalis* appears to employ a different strategy, spreading the reproductive effort over a longer period of time, which may attenuate severe intraspecific competition; this strategy allows the density of larvae, and new recruits in the population, to remain much lower than if breeding were condensed into a short period.

Obviously the larvae of late spawning species may find the suitable settlement sites occupied unless previous recruits are removed by, for example, predation or disease; this is therefore a high risk strategy if it relies only on chance. *S. clava* larvae appear to prefer to settle on a well-conditioned surface, i.e. a substratum with an established organic layer (Holmes, 1968). Spawning from late August to early September means that the *S. clava* larvae are settling when many annual macrophytes are dying back to reveal conditioned substratum available for settlement. Substratum exposure is particularly pronounced near the surface where annual chlorophyceæ die back, and this therefore represents a likely competitor-free zone for larval settlement late in the year. Thus it would appear that the strategy adopted by *S. clava* is to breed late in the year so that larvae can move in and settle near the surface after annual algae die back, avoiding direct competition with other solitary ascidian species. This raises the question "would other species recruit near the surface if the algae were removed?". This experiment was not attempted, but it seems unlikely that larvae of the other species would settle on the exposed substratum since larval recruitment on the surface panels was sparse in early summer, before *S. clava* started breeding. Furthermore, any *C. intestinalis* recruited near the surface would probably be killed by exposure to the higher intensity of UV radiation present (Jokiel, 1980).

Fouling panel experiments demonstrated recruitment zonation of two of the three species of solitary ascidians studied (Chapter 4), and evidence of this zonation was detected in the field observations of fouling communities reported by other workers (Mills, 1984; Collins & Mallinson, 1987). Dalby & Young (1992) attributed the similar recruitment zonation of *Styela plicata* on fouling panels to early post-settlement mortality, and Hurlbut (1991a) proposed juvenile mortality to explain the depth distribution of the colonial ascidian *Didemnum candidum*. But it was demonstrated in the present study, albeit with only a single experiment, that the zonation observed on fouling panels exposed in the Fawley inlet was not due to the presence of the panels *per se* or to other ecological factors, e.g. post-settlement mortality, affecting the survival of recruits at the particular depth of the panels in the water column (Chapter 14); however, it should be noted that any *C. intestinalis* larvae that settle near the surface may be killed by exposure to UV radiation (Jokiel, 1980), and therefore not be censused, unless they settle in a shaded environment (Schmidt & Warner, 1984). The distribution of the adults therefore appears to be a direct result of the selection of settlement site by the larvae, suggesting zonation of pre-settlement larvae in the water column. But is it feasible that larvae can select their settlement site?

Nelson (1928) proposed that larvae found suitable settlement sites by chance. He postulated that as larvae, which had been widely dispersed by currents during their planktonic phase, approached competence they were faced with a simple choice - settle or die. In discussing the settlement of larval Lamellibranchs, he suggested that "for those which through chance happened to 'fall upon good ground' there will be many more which through this same chance will 'fall by the way-side' and be destroyed". Colman (1933) supported the view that pelagic invertebrate larvae fell indiscriminately onto suitable and unsuitable substrata (like the seeds of terrestrial plants) and therefore had only a single, random chance of reaching a

suitable habitat for recruitment and development. But Wilson (1937) and Cole (1938) believed that the pelagic larvae of *Notomastus* and *Ostrea edulis* respectively, might be able to discriminate between favourable and unfavourable habitats, delaying metamorphosis if an unsuitable site was encountered. Thorson (1946) changed the emphasis from chance settlement to delayed settlement when he observed that "several, probably most, pelagic larvae have a certain space of time just before metamorphosis during which they may or may not metamorphose, according to the presence or absence of a substratum suitable for the young stage". The idea that marine larvae could remain pelagic until a suitable habitat was found for metamorphosis reinforced the view that they did not settle at random, but it was Wilson (1948, 1953, 1954), working with *Ophelia* larvae, who demonstrated that some invertebrate larvae were capable of selecting a substratum for settlement. Evidence to support this hypothesis has since been found in the settlement behaviour of many species of invertebrate larvae, see for example Crisp (1974;1976), Davis (1987), Durante (1991) and Hurlbut (1991b), so this phenomenon is not unusual and site-selection remains the current view of larval settlement behaviour for most species.

Much of the larval settlement behaviour described to-date involves the exploration of substrata, the "fine tuning" of settlement site selection, for example the crawling behaviour of *Balanus* cyprids (Walters *et al.*, 1996). Ascidian larvae were thought to lose any ability for settlement-site exploration once settlement had occurred (Crisp, 1976), but Davis (1987) observed the settlement behaviour of the larvae of the colonial ascidian *Podoclavella cylindrica* (Quoy & Gaimard) *in situ* and reported that they could detach from substrata after physically contacting them. Thus it seems likely that at least three stages are involved in larval settlement. In the first, larvae respond to gradients of physical factors in the water surrounding the settlement site so as to bring them into closer proximity to a suitable

substratum. In the second stage, gradients of waterborne chemical cues attract the larvae to the substratum surface; and in the final stage, the larvae make physical contact with the substratum and may carry out site exploration prior to metamorphosis. The present study focuses on the cues that could bring the larvae into the neighbourhood of the settlement site, the stage which fouling panel experiments indicated was likely to influence greatly the distribution of *A. aspersa* and *S. clava* recruits. The formation of zones of high larval concentration close to the settlement site could explain the distributions of these recruits. How could this larval zonation be achieved?

Settlement of invertebrate larvae may be initiated by chemical cues, e.g. ascidian tunic exudate (Durante, 1991) and other natural macromolecules (Roberts *et al.*, 1991) including γ-aminobutyric acid (Qian & Bryan, 1996; Dupré & Tapia, 1996), but it is unlikely that a chemical concentration gradient could persist far from source in the dynamic environment of a tidal harbour. Surface texture and the associated hydrography may also initiate settlement (Schmidt, 1982; Young, 1982), but only after the surface has been approached. Thus it seems likely that pre-settlement larval behaviour is influenced by physical factors, of which light, gravity and hydrostatic pressure are the most reliable (Chapter 1). How these cues can generate the larval behaviour necessary to produce the observed adult distributions will be deduced for each species from the results of the present study and other published work.

Ciona intestinalis

Spawning in *C. intestinalis* is controlled by a photoreceptor located at the top of the spermatoduct (Reese,1967) and normally occurs in the early morning (Berrill, 1947). In the present study, most eggs were produced in the early afternoon (Chapter 6) possibly due to

disturbance of the adults. Hatching was not synchronised, with the first larvae appearing approximately 12 hours after spawning but some larvae still hatching up to 12 hours later. Although there is a wide range of time estimates, it is apparent that the eggs will be in the water column for a minimum of 12 hours.

The eggs of *C. intestinalis* were negatively buoyant (Chapter 7), sinking in still water despite the presence of projections. There was no indication that the buoyancy of eggs varied with development stage, therefore it is reasonable to assume hatching occurs at depth and the larval phase starts with exposure to some degree of increased hydrostatic pressure. Larvae remained active for at least 18 hours after spawning, so they were active throughout the daylight period. The larvae were negatively buoyant (Chapter 7). Increase in hydrostatic pressure did not appear to influence the proportion of larvae exhibiting negative buoyancy. No evidence could be found to suggest that *C. intestinalis* larvae can regulate buoyancy directly by morphological or physiological mechanisms, so the effect of negative buoyancy can be modified only by behaviourally-mediated locomotor responses. Downward movement may be effected by passive sinking or active locomotion; upward movement, however, can be achieved only by active locomotion. Recently hatched larvae were more buoyant than mature larvae; the decline in buoyancy was probably due to depletion of lipid reserves, and consequent increase in specific gravity, as the lecithotrophic larvae expend energy swimming.

A substantial proportion of active *C. intestinalis* larvae rose up in the vertical behaviour chamber in absence of light (Chapter 8). This vertical movement opposed and exceeded the effect of negative buoyancy and therefore represented an active negative geotactic response; larval distribution in the horizontal chamber changed little in absence of light, confirming the

vertical movement as a directed population response. The proportion of the population of *C. intestinalis* larvae that exhibited active negative geotaxis declined with increase in applied hydrostatic pressure to a minimum at about 2 m head of water, then increased with hydrostatic pressure to at least 3.5 m head of water (Chapter 11). The formation of a circulation cell in the water column below 2 m depth was proposed. Since gravity is a constant force, high barokinesis was proposed to explain this change in response with upward movement directed by the negative geotactic response of the larvae and downward movement resulting from passive sinking when the active motion ceased.

As the larval phase occurs during the hours of daylight, light is a potential cue for *C. intestinalis* larvae. When movement was restricted to the horizontal plane, with no applied pressure, young *C. intestinalis* larva exhibited positive phototaxis whereas mature larvae exhibited negative phototaxis (Chapter 9), the proportion of the population moving towards and away from the light source, respectively, increasing with light intensity. This suggests that an ontogenetic change is occurring.

Ontogeny of phototaxis is not uncommon in invertebrate larvae; it has been reported in crab larvae (Forward & Costlow, 1974; Bigford, 1979), nudibranch larvae (Miller & Hadfield, 1986), polychaete larvae (Young & Chia, 1982) and many ascidian larvae including *C. intestinalis* larvae (Berrill, 1947; Millar, 1953; Dybern, 1963). However, although the morphology of the photoreceptor systems of ascidian larvae (Dilly, 1961) and the ultra-structure of differentiating (Barnes, 1974) and fully differentiated larval ocelli (Dilly, 1964; Eakin & Kuda, 1971; Barnes, 1971) have been widely studied, only Kajiwara & Yoshida (1985) have related ontogenic changes in light response to changes in larval ocellar structure. They reported that newly hatched *Ciona savignyi* Herdman larvae were

negatively geotactic and indifferent to light, but the larvae exhibited a shadow response after one and a half hours, weak negative phototaxis after two and a half hours and strong negative phototaxis after three and a half hours. They observed that in newly hatched larvae the flat pigment cell of the ocellus contained sparsely scattered pigment granules and several irregularly arranged tubular membranes. One hour after hatching the pigment cell became roughly L-shaped, and the tubular membranes became paddle shaped and increased in both size and number. After three and a half hours the ocellus became fully differentiated with the paddle-shaped membranes arrange into lamellae. Kajiwara & Yoshida (1985) concluded that changes in photic behaviour coincided with the course of differentiation of the ocellar elements. It is possibly that similar ontogenetic changes occurred in young *Ciona intestinalis* in this study and that the effect of light intensity was superimposed on these ontogenetic changes, confusing interpretation of the experimental results.

Comparison of the larval distributions observed when populations were allowed to respond to gravity in the presence (Chapter 10) and absence (Chapter 8) of light suggests that there may be two groups of mature larvae with opposite phototactic responses in the population. When light was applied from above, the population phototactic response changed from positive to negative at around 500 lux, then changed back to positive by 1000 lux, but no change in phototactic response was observed in the limited experiments with light from below. This is unlikely to be an ontogenetic change in phototactic response because the comparison was made between randomly aged mature larvae.

Although *C. intestinalis* larvae exposed to a light flux of 250 lux in the vertical chamber exhibited a similar distribution with hydrostatic pressure to that observed in the absence of light (Chapter 12), an active phototactic response was observed as the light flux increased

to 1500 lux. Thus larvae hatched in low light conditions at depth will tend to rise in the water column as a result of negative geotaxis, supplemented by positive phototaxis as the light intensity increases towards the surface. If light intensities decrease to around 500 lux larvae will tend to sink until the increased pressure and low light level elicits upward movement again, forming a circulation cell.

The laboratory experiments indicate that *C. intestinalis* larvae do not concentrate at any specific depth but circulate in the water column, possibly within a circulation cell formed below 2 m depth, until other cues promote settlement. Recruitment experiments indicated patchy distribution through the water column with the majority of animals occurring below 1.5 m depth. The diffuse distribution of larvae in the water column, coupled with the long reproductive season, suggests that the *C. intestinalis* larva is an opportunistic settler. Few larvae were collected when the water column was sampled at different depths (Chapter 14), but the observed distribution did not contradict the proposed hypothesis.

How could the dense populations of adult *C. intestinalis* observed around Southampton Water develop if the larvae do not concentrate in pre-settlement zones? The larvae could be attracted to established colonies by a chemical cue excreted by established adults and juveniles, but such cues would only be effective over short distances. An alternative explanation may be deduced from the work of Svane and Havenhand (1989), who reported that eggs could be either freely spawned by *C. intestinalis* or released in strings of mucus which were adhesive and readily adhered to the adults. They also observed that newly hatched larvae could be retained in the string of mucus until settlement occurred. The dense aggregations of *C. intestinalis* observed in the field could therefore be explained by abbreviated dispersal due to 'mucus-string' spawning and epibenthic retention of eggs. No

strings of eggs were observed in the spawning tanks in this study, but the eggs were not examined until after filtration, a process which would probably have disrupted any mucus-strings present.

Overhangs and other shaded habitats are frequently colonised by ascidians (Millar, 1971; Gulliksen, 1972; Goodbody, 1974; Berrill, 1975; Olson, 1983; Young & Chia, 1984) and it has often been assumed that these habitats are located by negative phototaxis (Dybern, 1963; Thorson, 1964; Crisp & Ghobashy, 1971; Millar, 1971), but Young & Chia (1985) demonstrated that the shadow response (Grave, 1920; Mast, 1921) could not explain larval selection for these settlement sites. Many authors have reported *C. intestinalis* populations on the under-side of ledges or the roofs of sub-marine caves (e.g. Dybern, 1963). Recruitment on substrata in these positions is consistent with the negative geotactic response of *C. intestinalis* larvae observed at hydrostatic pressures greater than 2.5 m head of water and low light intensity. However, it is possible that, rather than selecting shaded habitats, larvae settle indiscriminately but those that settle on exposed substrata near the surface are killed by exposure to UV radiation (Jokiel, 1980) before the recruits are censused. Schmidt & Warner (1984) showed that the caging of approximately 1 m deep settlement panels dramatically increased the recruitment of *C. intestinalis* and suggested that this was due to reduced light intensity rather than reduced predation.

Ascidiella aspersa

Ascidiella aspersa spawned in the late afternoon and the larvae hatched about mid afternoon on the following day. The eggs are therefore in the water column for at least 18 hours. The eggs were negatively buoyant in still water, but this may be because the follicle

cells were damaged during the filtering process (Lambert & Lambert, 1979; C. Young, pers. comm.), so it is uncertain where hatching is likely to occur in the water column. The larvae were active for at least seven to eight hours and were therefore probably competent towards the end of the daylight period. These data indicate that the egg is probably a more important distribution phase than the larva.

A. aspersa larvae were negatively buoyant. However, the proportion of larvae exhibiting negative buoyancy decreased as hydrostatic pressure increased, reaching a minimum at 2 m head of water, then increased again as the pressure increased further. No explanation could be offered for this buoyancy anomaly, but it is unlikely to be an artefact associated with the experimental protocol because a similar trend in distribution was noted for active larvae in the absence of light (Chapter 8). It was proposed that it would be energetically favourable for *A. aspersa* larvae to float at a hydrostatic pressure of about 2 m head of water, and therefore a large proportion of the larval population would accumulate at this depth. Recently hatched larvae were more buoyant than mature larvae; the decline in buoyancy was probably due to depletion of the lipid reserves of the lecithotrophic larvae.

A substantial proportion of active *A. aspersa* larvae rose up in the vertical behaviour chamber in absence of light (Chapter 8). This vertical movement, which exceeded the effect of negative buoyancy, represented an active negative geotactic response. An enhanced response was observed for recently hatched larvae. Larval distribution in the horizontal chamber changed little in absence of light, confirming the vertical movement as a directed population response. The proportion of the population of *A. aspersa* larvae that exhibited active negative geotaxis increased with applied hydrostatic pressure to a maximum at about 2 m head of water (Chapter 11). At hydrostatic pressures greater than 2.5 m larvae tended

to sink, suggesting a change from high barokinesis to low barokinesis. This pattern of larval distribution with applied hydrostatic pressure is similar to that observed for anaesthetised larvae.

A small proportion of mature *A. aspersa* larvae exhibited positive phototaxis when movement was restricted to the horizontal plane; the proportion did not vary with light intensity and was insufficient for the it to be considered a characteristic phototactic response. *A. aspersa* larvae also exhibited positive phototaxis when vertical movement was allowed and light was applied from above; the minimum response occurred at a light flux of around 500 lux. When light was applied from below, the distribution of larvae in the chamber was similar to that observed in the absence of light, indicating that there was no negative phototactic response in the population and that the positive phototactic response was weaker than the negative geotactic response.

Larvae hatched at depth in low light will tend to rise in the water column by negative geotaxis; in so doing they will be exposed to reduced pressure and increased light intensity and, at a hydrostatic pressure of about 2 m, a large proportion will tend to sink again to complete a cycle in a circulation cell. Larvae that escape from this cycle will rise further in the water column and be exposed to reduced pressure and increased light intensity, causing them to sink again to rejoin the circulation cell. These results suggest that *A. aspersa* larvae may circulate in the water column at depths below 2 m. The buoyancy experiments indicated that it would be energetically favourable for the larvae to hold station at a depth of around 2 m, so they may spend more time at this depth that at other points in the circulation cell. Thus larvae may tend to concentrate at this depth, the depth at which the initial intense

recruitment occurs. Insufficient larvae were collected during the sampling of the water column to support this hypothesis, although the observed distribution did not contradict it.

Hydrostatic pressure is an important factor in determining settlement depth of *A. aspersa* larvae, but it does not operate in isolation; it provides the impetus for motion which is directed by the behavioural response to light. The importance of light intensity in determining the upper limit of recruitment on submerged substrata was identified by Schmidt & Warner (1984) who showed that the caging of approximately 1 m deep settlement panels dramatically increased the recruitment of *A. aspersa* and suggested that this was due to reduced light intensity rather than reduced predation.

Styela clava

Styela clava spawned in the early evening and the larvae hatched about mid morning on the following day. The eggs were negatively buoyant in still water. There was no indication that the buoyancy of eggs varied with development stage, therefore it is reasonable to assume hatching occurs at depth and the larval phase starts with exposure to some degree of increased hydrostatic pressure. The larvae were active for at least ten hours, as reported by Na & Lee (1977), and were therefore probably competent through the mid-afternoon to evening daylight period. These data indicate that the egg is probably a more important distribution phase than the larva.

S. clava larvae were negatively buoyant, the proportion of larvae exhibiting negative buoyancy increasing with hydrostatic pressure; so it was energetically favourable for many

of these larvae to remain near the surface where negative buoyancy generated minimum effect. Although buoyancy may aid larvae to maintain position near the surface, movement through the water column can only be achieved by active locomotor responses.

Over 70% of young *S. clava* larvae, and approximately half of the population of mature larvae, were found in the top section of the vertical behaviour chamber irrespective of the applied hydrostatic pressure (Chapter 11), indicating that the negative geotactic response of these larvae is not affected by pressure increase but declines with age. This change is unlikely to be due to ontogeny of the response to gravity since it involves a reduction in the population response rather than a complete transition from geonegative to geopositive behaviour, as reported for *Cancer irroratus* larvae (Bigford, 1979) and *Ebalia tuberosa* larvae (Schembri, 1982). As the vertical movement of *S. clava* larvae exceeded the effect of negative buoyancy, it must represent an active negative geotactic response. Larval distribution in the horizontal chamber changed little in absence of light, confirming the vertical movement as a directed population response.

In the absence of applied hydrostatic pressure, mature *S. clava* larvae exhibited negative phototaxis when movement was restricted to the horizontal plane. The proportion of the population moving away from the light source increased with the applied light intensity, reaching approximately 40% at 2000 lux, so this response can be considered characteristic. But when hydrostatic pressure of 2.5 m was applied, *Styela clava* larvae exhibited positive phototaxis, the proportion of the population moving towards the light source increasing with the applied light intensity, reaching approximately 48% at 1500 lux. This change in distribution appears to be the result of high barokinesis. The difference in the distribution of larvae exposed, in the absence of light, to hydrostatic pressure of 2.5 m compared with that

found with no applied hydrostatic pressure indicated that motion has been initiated by the introduction of hydrostatic pressure. The motion is random in the absence of light, but manifests as positive phototaxis when a light flux of 500-1500 lux is applied. Photokinesis can be excluded as it would not produce increased larval movement in the absence of light. The results indicate that when the effect of gravity is minimised, application of hydrostatic pressure causes a change in the population phototactic response of *S. clava* larvae. The proportion of the population exhibiting positive phototaxis at light intensities of between 1000 and 1500 lux exceeds 30%, thus this response can be considered characteristic.

In the absence of applied hydrostatic pressure, mature *S. clava* larvae also exhibited negative phototaxis when movement was permitted in the vertical plane and light was applied from above. The maximum response occurred with a light flux of around 250 lux. But when hydrostatic pressure was applied to this experimental system, large proportions of larvae congregated in the top section of the chamber; the minimum response occurred at a light flux of around 250 lux. This larval re-distribution was a positive phototactic response because the proportions of the population in the top section exceeded those recorded in the absence of light. The maximum response was observed with an applied light flux of 1000 lux and hydrostatic pressures between 2 and 3 m. Attenuation of positive phototaxis occurred with an applied light flux of greater than 1000 lux and hydrostatic pressures less than 2.5 m head of water.

S. clava larvae hatched at depth in low light conditions, or the absence of light, will tend to rise in the water column as a result of negative geotaxis; in so doing they will be exposed to increased light intensity and, at a light intensity of about 250 lux, a proportion of the population will tend to sink again. Those that pass through this zone continue to rise and

are exposed to increasingly higher light intensities which induce positive phototaxis. The magnitude of the phototactic response declines as hydrostatic pressure decreases, so a proportion of the larvae will begin to sink as they near the surface. But as they sink, the larvae encounter conditions of light flux and pressure that elicit an increased positive phototactic response and the larvae rise again. This circulation cell keeps a large proportion of the population of *S. clava* larvae in the vicinity of the surface where they will eventually settle. Larvae exposed to a light intensity of about 250 lux will tend to sink and this may account for the smaller proportion of adults observed at depths of 2 m or more.

Table 163 Summary of the responses of ascidian larvae to physical cues.

Physical cue or combination	Response of mature larvae		
	C. intestinalis	*A. aspersa*	*S. clava*
Gravity	-ve geotaxis	-ve geotaxis	strong -ve geotaxis*
Gravity + pressure	-ve geotaxis, minimum at 2 m	-ve geotaxis, maximum at 2 m	no change in strong -ve geotactic response
Light	-ve phototaxis	weak +ve phototaxis	-ve phototaxis
Light + pressure	Not tested	Not tested	+ve phototaxis, high barokinesis*
Gravity + light	+ve phototaxis to 500 lux -ve phototaxis above 500lux	+ve phototaxis, minimum response at 500 lux	-ve phototaxis, maximum response at 250 lux
Gravity + light + pressure	formation of a circulation cell	formation of a circulation cell*	+ve phototaxis, min. response at 250 lux

* important factors leading to larval zonation

The larvae of these three ascidian species appear to react very differently to changes in light flux and applied hydrostatic pressure (Table 163). Whilst I have offered behavioural explanations for the observed distributions based on phototaxis, geotaxis and barokinesis, it should be noted that the pressure and light flux changes were discrete and static, i.e. the experiments determined the distribution of larvae rapidly exposed to a specific hydrostatic pressure and light flux, then held at those levels for an hour at constant temperature. Under natural conditions, the pressure and light ranges are continuous and changes are dynamic,

i.e. pressure and light intensity change as the larvae sink or rise, so feedback control of depth may be possible, as proposed for crab larvae by Sulkin (1984) and Forward (1989). I have attempted to translate the results of a limited number of static experiments into a dynamic qualitative model of larval behaviour for each of the species studied; extrapolation from spot observations to a dynamic continuum is always problematic, so the interpretations must be considered tentative. Nevertheless, it is apparent that the distributions of *C. intestinalis* and *A. aspersa* larvae, but not *S. clava* larvae, change in a metre column of water when the applied hydrostatic pressure is increased beyond 2 m head of water.

Where *S. clava* is not present, no other species of solitary ascidian occupies the habitat just exposed at, and immediately below, low water in harbours and sheltered rocky coves although other organisms, e.g. barnacles and mussels, are present. It would therefore appear that *S. clava* is not competing directly against indigenous ascidian species or displacing them when it colonises substrata near the surface, but is filling an unexploited ascidian niche; non-ascidian competition was not considered in this study. Thus *S. clava* is a successful immigrant because it can minimise competition by exploiting a niche not available to other ascidians. What strategies are involved in exploiting this niche? Essentially the larvae must be attracted towards the surface when shallow settlement substrata are available. This is achieved by autumn production of larvae that exhibit high barokinesis with motion directed in a negatively geotactic and positively phototactic direction. The short life span of these larvae may account for the limited spread of *S. clava*.

C. intestinalis appears to be an opportunist. Its reproductive and settlement strategy appears to be selected to maximise the temporal and spatial availability of larvae, thus increasing the probability of a larva finding a settlement site. The temporal component of

the strategy consist of multiple spawning through a long season so that larvae are in the water most of summer, ready to settle in any vacant niche that arises due, for example, to the death of the incumbent. The relatively long-lived larvae then circulate in the water column in response to the combined effects of gravity light and hydrostatic pressure, exposing them to a wider range of potential settlement sites than larvae that are concentrated in zones; this is the spatial component of the strategy.

The spawning and settlement season of *A. aspersa* is a more intense than that of *C. intestinalis* and earlier than that of *S. clava*. Its reproductive and settlement strategy appears to be to concentrate some larvae in a zone and saturate suitable substrata with them over a short period of time, probably excluding competing larvae in the process. Early summer spawning may permit the larvae to colonise substrata cleared by winter storms and deaths. The interaction of larval buoyancy, phototactic and geotactic responses, modulated by barokinesis, offers an explanation for the increased concentration of *A. aspersa* larvae at a depth of about 2 m, but it does not account for the initial intense, almost exclusive, recruitment observed at that depth. This suggests that there is another factor involved. Nevertheless, the interaction of the cues examined in this study forms an important part of the zonation mechanism; this interaction would not produce such intense zonation of pre-settlement *A. aspersa* larvae as *S. clava* larvae, which may account for the eventual wide-spread recruitment of *A. aspersa*.

The effect of hydrostatic pressure has often been neglected in studies of the responses of invertebrate larvae to physical cues. Barokinesis plays an important role in determining the behavioural response of the ascidian larvae examined in this study, but is this hydrostatic

pressure likely to be of general importance to larvae in shallow waters? Knight-Jones & Morgan (1966) considered it likely that many planktonic animals regulated their depth through pressure responses. As depth-regulating mechanisms operate independently of light, they thought that pressure responses would be particularly useful where turbidity restricted light penetration, and they noted that these responses were conspicuous in planktonic animals from shallow inshore waters, which are often turbid.

Circulation cells have been proposed for *C. intestinalis* and *A. aspersa* larvae in the present study, but the effect of repeated exposure to pressure change and the possibility of habituation have not been considered. In many organisms, the initial pressure change evokes a response more readily than subsequent changes, and larger pressure changes tend to reduce the organisms susceptibility to smaller ones (Knight-Jones & Morgan, 1966). Rice (1964; 1966) reported considerable variation in the readiness with which animals responded to pressure changes, not only between species, but between individuals and between different physiological states of the same individual. He concluded that "the incidence of depth-regulatory swimming excursions in response to small pressure changes depends upon how recently the animals concerned have previously exerted themselves in that way". Thus attenuation of response, if not habituation, is likely if the larval circulation cycle is repeated too rapidly. A slow circulation is also preferable because it is probable that the pressure responses of many planktonic animals will not be invoked by brief pulses (Knight-Jones & Morgan, 1966); rapid accommodation, i.e. a short latent period, is unusual in planktonic animals. Finally, is it realistic for the circulation cell to rely on larvae passively sinking on the downward leg? Knight-Jones & Qasim (1955, 1966) considered that planktonic animals which were capable of sinking quickly generally responded to falling pressure by inactivity, i.e. they exhibited high barokinesis, whereas photonegative or geopositive swimming was

typical of animals which did not sink quickly because they were small or buoyant. The larvae of all three ascidian species were negatively buoyant, so passive sinking would appear to be a reasonable mode of descent in the water column for all of them.

Is it reasonable to postulate an ascidian larval circulation cell driven by the response to hydrostatic pressure? Most crustacian larvae are sensitive to changes in pressure at some developmental stage and, in general, an increase in pressure induces an increase in swimming speed (high barokinesis), negative geotaxis and positive phototaxis (Rice, 1964; Knight-Jones & Morgan, 1966; Morgan, 1984; Sulkin, 1984). Alternatively, a pressure decrease causes a descent of the crustacian larvae due to passive sinking (Jacoby, 1982; Schembri, 1982). Forward (1989) incorporated these responses into a negative feedback model of crustacian larval depth regulation that is similar to the circulation cell proposed for the ascidian larvae.

One factor that has not been examined in this study is the effect of UV-radiation on recruitment. UV radiation penetrates clear ocean water almost as effectively as visible light (Smith & Baker, 1979), but the dissolved and particulate organic matter in estuarine and coastal waters attenuates the penetration. Young (1982) showed that the response of some ascidian larvae to light is wavelength dependent. It is possible that the larvae of *C. intestinalis*, *A. aspersa* and *S. clava* respond differently to UV radiation, producing or reinforcing larval zonation. But it is more likely that juvenile recruits of species that are not UV-tolerant (adapted to cope with high levels of ultraviolet light exposure) are rapidly killed by it. Jokiel (1980) showed that adult specimens of *C. intestinalis* exposed to natural sunlight were killed within 13 days, whereas specimens protected by a UV-filter survived.

This may explain why *C. intestinalis* is rarely found near the surface; larval response to light and gravity ensures that the majority of larvae settle a safe distance below the surface; those that do not are killed by exposure to UV radiation, unless recruited in a shaded habitat.

Some ascidian species appear to be adapted to deal with exposure to UV-radiation; *Ascidia interrupta*, which contains a dense black pigment that is thought to act as a UV absorber (Endean, 1961), is found in high light environments where *C. intestinalis* does not occur (Jokiel, 1980). It is possible that the grey coloration of *A. aspersa* confers some UV protection on this species and allows it recruit closer to the surface than *C. intestinalis*. It would then follow that the brown pigmentation and thick test of *S. clava*, coupled with the dense layer of epibionts usually associated with the adults, are probably crucial for the recruitment of this species close to the surface.

Settlement-site selection by larvae permits sessile animals to exploit specialised habitats, thus reducing competition and aiding the spread of the species. Since the larvae select the site for adult growth and development, larval choices must affect recruitment success. Therefore the ability of larvae to find a suitable habitat based on physical, chemical or biotic cues, should be subject to selective pressure and have a genetic basis. This means that any increase in adult fitness that can be attributed to larval choice may cause heritable components of larval behaviour responses to be retained in the population over evolutionary time. The possibility of a heritable behaviour raises the question "Are the populations of *S. clava* living at the surface and at depth genetically different?". Unfortunately the responses to physical cues of larvae from the two populations were not compared in the present study.

16 REFERENCES

Abbott, D.P. & Johnson, J.V., 1972. The ascidians *Styela barnharti, S plicata, S. clava,* and *S montereyensis* in Californian waters. *Bull. South Calif. Acad. Sci.*, 71, 95-105.

Bainbridge, R., 1961. Migration. In: *Physiology of Crustacea,* (ed. T. H. Waterman). Vol. II, Chapter 12, pp. 431-463. Academic Press, New York.

Barnes, H., 1959. Sea surface temperatures at Millport. *J. mar. biol. Ass. U.K.*, **38**, 423-424.

Barnes, R.S.K., Coughlan, J. & Holmes, N.J., 1973. A preliminary survey of the macroscopic bottom fauna of the Solent, with particular reference to *Crepidula fornicata* and *Ostrea edulis. Proc. Malac. Soc. Lond.*, 40, 253-275.

Barnes, S.N., 1971. Fine structure of the photoreceptor and cerebral ganglion of the tadpole larva of *Amaroucium constellatum* (Verrill). (Subphylum: Urochordata; Class: Ascidiacea). *Z. Zellforsch.*, 117, 1-16.

Barnes, S.N., 1974. Fine structure of the photoreceptor of the tadpole larva during development. *Cell Tissue Res,*. 155, 27-45.

Bayne, B.L., 1964. The responses of the larvae *Mytilus edulis* L. to light and to gravity. *Oikos*, 15, 162-174.

Begon, M., Harper, J.L. and Townsend, C.R., 1990. Ecology. Individuals, Populations and Communities (2nd ed.). Oxford: Blackwell Scientific Publications.

Berrill, N.J., 1928. The identification and validity of certain species of ascidians. *J. mar. biol. Ass. U.K.*, 15, 159-175.

Berrill, N.J., 1935. Studies in Tunicate development. III. Differential retardation and acceleration. *Phil, Trans. Roy. Soc. London*, B, CCXXV, 255-336.

Berrill, N.J., 1947. The Development and Growth of *Ciona. J. mar. biol. Ass. U.K.*, **26**, 616-625.

Berrill, N.J., 1948. Budding and the reproductive cycle of *Distaplia. Q. J. Microsc. Sci.*, **89**, 253-9.

Berrill, N.J., 1950. The Tunicata. The Ray Society, London. 354pp.

Berrill, N.J., 1975. Chordata: Tunicata. In: *Reproduction of Marine Invertebrates, Vol II,* edited by A. C. Giese & J. S. Pearse, Academic Press, New York, 241-282.

Bigford, T.E., 1979. Ontogeny of light and gravity responses in rock crab larvae *(Cancer irroratus). Mar. Biol.*, 52, 69-76.

Boer, den P.J., 1986. The present status of the competitive exclusion principle. *Tree* 1(1), 25-28.

Bone, Q., 1992. On the locomotion of ascidian tadpole larvae. *J. mar. biol. Ass. U.K.*, **72**, 161-186.

Branford, J.R., 1978. The influence of day length, temperature and season on the hatching rhythms of *Homarus gammarus*. *J. mar. biol. Ass. U.K.*, **58**, 639-648.

Brown, J.S., 1989. Coexistence on a seasonal resource. *Am. Nat.*, **133**, 169-182.

Bruun, A.F., Greve, S., Mielche, H. & Sparch, R., ed. (1956). *The Galathea Deep Sea Expeditions, 1950-52*. Macmillan, New York.

Buizer, D.A.G., 1980. Explosive development of *Styela clava* Herdman, 1882, in the Netherlands after its introduction. *Bull. Zool. Mus. Univ. Amsterdam*, **7**, 181-5.

Butler, A.J., 1986. Recruitment of sessile invertebrates at five sites in Gulf St. Vincent, South Australia. *J. Exp. Mar. Biol. Ecol.*, **97**, 13-36.

Cameron, R.A. & Rumrill, S.S., 1982. Larval abundance and recruitment of the sand dollar *Dendraster excentricus* in Monterey Bay, California, USA. *Mar. Biol.*, **71**, 197-202.

Carlisle, D.B., 1951. On the hormonal and neural control of the release of gametes in ascidians. *J. Exp. Biol.*, **28**, 463-472.

Carlisle, D.B., 1954. *Styela mammiculata* n.sp., a new species of ascidian from the Plymouth area. *J. mar. biol. Ass. U.K.*, **33**, 329-334.

Chengxing, Z., 1988. The ascidians among the fouling organisms in the coast of the Yellow Sea and Bohai Gulf. *Acta. Zool. Sin. Dongwu Xuebao*, **34**, 180-188. (in Chinese).

Christiansen, J. & Thomsen J.C., 1981. *Styela clava* Herdman, 1882, a species new to the Danish fauna (Tunicata Ascidiacea). *Steenstrupia*, **7**, 15-24.

Clarke, G.L. & Denton, E.J., 1962. Light and Animal Life. In: *The Sea*, (ed. M.N. Hill). Vol. 1, Chapter 10, pp. 456-468. Interscience Publishers, London.

Cloney, R.A., 1978. Ascidian metamorphosis: review and analysis. In: *Settlement and Metamorphosis of Marine Invertebrate Larvae*, edited by F. -S. Chia & M.E. Rice, Elsevier North-Holland Biomedical Press, Amsterdam. p.255-282.

Cloney, R.A., 1987. Phylum Urochordata, Class Ascidiacea. In: *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*, edited by M.F. Strathmann, University of Washington Press, Seattle. p.607-63 9.

Cole, H.A., 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. *J. mar. biol. Assoc. U.K.*, **22**, 469-484.

Collins, K.J., 1978. The fluxes of organic carbon and nutrients in Southatnpton Water. PhD thesis, University of Southampton.

Collins, K.J. & Mallinson, J.J., 1987. Marine flora and fauna of Southampton Docks. Report to the Nature Conservancy Council. Contract No: HF3-11-52(7).

Colman, J., 1933. The nature of the intertidal zonation of plants and animals. *J. mar. biol. Ass. U.K.*, **18**, 435-476.

Connell, J.H., 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecolog.*, **42**, 710-723.

Connell, J.H., 1980. Diversity and the co-evolution of competitors, or the ghost of competition past. *Oikos*, **35**, 131-138.

Connell, J.H., 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. *J. Exp. Mar. Biol. Ecol.*, **93**, 11-45.

Costello, D.P., Davidson, M.E., Eggers, A., Fox, H.M. & Henley, C., 1957. Methods for obtaining and handling marine eggs and embryos. Mar. Biol. Lab. Woods Hole, Mass. 247 pp.

Coughlan, J., 1969. The leathery sea squirt - a new ascidian from Milford Haven. *Nature Wales*, **11**, 192-193.

Coughlan, J., 1985. Occurrence of the immigrant ascidian *Styela clava* Herdman in Heysham Harbour, Lancashire. *Porcupine Newsletter*, **3**, 85-87.

Coughlan, J. & Fleming, J.M., 1978. A versatile pump-sampler for live zooplankton. *Estuaries*, **1**, 132-135.

Crisp, D.J., 1974. Factors influencing the settlement of marine invertebrate larvae. In: *Chemoreception in Marine Organisms*. ed. P.T.Grant & A.M.Mackie. Academic Press, New York and London. p.177-265.

Crisp, D.J., 1976. Settlement responses in marine organisms. In: *Adaptions to Environment: essays on the physiology of marine animals*, ed. R.C.Newell. Butterworth & Co., London. pp. 83-124.

Crisp, D.J. & Ghobashy, A.F.A.A., 1971. Responses of the larvae of *Diplosoma listerianum* to light and gravity. In: *Proceedings of the Fourth European Marine Biology Symposium*. ed. D.J.Crisp, Cambridge University Press. Cambridge. p.443-465.

Dalby, J.E. & Young, C.M., 1992. Role of early post-settlement mortality in setting the upper depth limit of ascidians in Florida epifaunal communities. *Mar. Ecol. Prog. Ser.*, **80**, 221-228.

Dauvin, J.C., Iglesias, A. & Gentil, F., 1991. Nouvelles espèces pour la Faune Marine de Roscoff - Crustacés Amphipodes, Cumacés et Décapodes, Mollusques Gastéropodes et Ascidies. *Cah. Biol. Mar.*, **32**, 121-128. (in French).

Davis, H.C. & Hidu, H., 1969. Effects of turbidity-producing substances in sea water on eggs and larvae of three species of bivalve molluscs. Veliger, **11**, 316-323.

Davis, A.R., 1987. Variation in recruitment of the subtidal colonial ascidian *Podoclavella cylindrica* (Quoy & Gaimard): the rôle of substratum choice and early survival. *J. Exp. Mar. Biol. Ecol.*, **106**, 57-71.

Davis, A.R. & Butler, A.J., 1989. Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Sluiter: evidence for closed populations. *J. Exp. Mar. Biol. Ecol.*, **127**, 189-203.

Davis, M.H., 1980. A standardized method for measurement of primary production. CERL Laboratory Report No. RD/LN/N211/79. Central Electricity Research Laboratories, Leatherhead.

Davis, M.H., 1983a. The effect of chlorination on entrained phytoplankton at three coastal power stations. CERL Laboratory Report No. TPRD/L/2468/R83. Central Electricity Research Laboratories, Leatherhead.

Davis, M.H., 1983b. The response of entrained phytoplankton to chlorination at a coastal power station (Fawley, Hampshire). CERL Laboratory Note No. TPRD/L/2470/N83. Central Electricity Research Laboratoiies, Leatherhead.

Digby, P.S.B., 1967. Pressure sensitivity and its mechanism in the shallow marine environment. *Symp. zool. Soc. Lond.*, 19, 159-188.

Dilly, P.N., 1961. Electron microscope observations of the receptors in the sensory vesicle of the ascidian tadpole. *Nature*, **191**, 786-787.

Dilly, P.N., 1962. Studies on the receptors in the cerebral vesicle of the ascidian tadpole. 1. The otolith. *Q. J. Micr. Sci.*, **103**, 393-398.

Dilly, P.N., 1964. Studies on the receptors in the cerebral vesicle of the ascidian tadpole. 2. The Ocellus. *Q. J. Micr. Sci.*, **105**, 13-20.

Dilly, P.N., 1969. The ultrastructure of the test of the tadpole larva of *Ciona intestinalis*. *Z. Zellforsch Mikrosk. Anat.*, **95**, 331-346.

Dupré, E. & Tapia, C., 1996. Settlement behaviour and metamorphosis induction in the scallop *Argopecten purppratus*. Poster presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966.

Durante, K.M., 1991. Larval behaviour, settlement preference, and induction of metamorphosis in the temperate solitary ascidian *Molgula citrina* Alder & Hancock. *J. Exp. Mar. Biol. Ecol.*, **145**, 175-187.

Duyl, F.C. van, Bak, R.P.M. & Sybesma, J., 1981. The ecology of the tropical compound ascidian *Trididemnum solidum*. 1. Reproductive strategy and larval behaviour. *Mar. Ecol. Prog. Ser.*, **6**, 35-42.

Dybern, B.I., 1963. Biotope choice in *Ciona intestinalis* (L.). Influence of light. *Zool. Bidr. Uppsala*, **39**, 589-601.

Dybern B.I., 1965. The life cycle of *Ciona intestinalis* (L.) f typica in relation to environmental temperature. *Oikos*, **16**, 109-131.

Dyer, K.R., 1970. *Some aspects of coastal and estuarine sedimentation*. Ph.D. thesis, University of Southampton.

Eakin, R.M. & Kuda, A., 1971. Ultrastructure of sensory receptors in ascidian tadpoles. *Z Zellforsch. Mikrosk. Anat.*, **112**, 287-312.

Endean, R., 1961. The test of the ascidian *Phallusia mammillata*. *Q. J. Microsc. Sci.*, **102**, 107-117.

Forward, R.B., Jr., 1989. Depth regulation of larval marine decapod crustaceans: test of an hypothesis. *Mar. Biol.*, **102**, 195-201.

Forward, R.B., Jr., & Costlow, J.D., Jr., 1974. The ontogeny of phototaxis by larvae of the crab *Rhithropanopeus harrisii*. *Mar. Biol.*, **26**, 27-33.

Forward, R.B., Jr., & Cronin, T.W., 1979. Spectral sensitivity of larvae from intertidal crustaceans. *J. Comp. Physiol.*, **133**, 311-315.

Fraenkel, G.S. & Gunn, D.L., 1961. The Orientation of Animals. Dover Publications, New York. 361 pp.

Gaines, S. & Roughgarden, J., 1985. Larval settlement rate: a leading determinant of structure in an ecological community of the marine intertidal zone. *Proc. Natl. Acad. Sci. USA*, **82**, 3707-3711.

Gaines, S., Brown, S. & Roughgarden, J., 1985. Spatial variation in larval concentrations as a cause of spatial variation in settlement for the barnacle, *Balanus glandula*, *Oecol.*, **67**, 267-272.

Gause, G. F., 1934. The struggle for existence. Hafner Publishing Company. (Reprinted 1964). 163pp.

Georges, D., 1971. La lumiere et le declenchement de la ponte chez *Ciona intestinalis*. In: *Proceedings of the Fourth European Marine Biology Symposium*, edited by D.J.Crisp, Cambridge University Press, Cambridge, pp. 561-569.

Giese, A.C. & Pearse, J.S., 1974. Introduction: General Principles. In: *Reproduction in Marine Invertebrates. Vol. 1*, edited by A. C. Giese, & J. S. Pearse. Academic Press. New York. pp. 1-49.

Goodbody, I., 1963. The biology of *Ascidia nigra* (Savigny). II. The development and survival of young ascidians. *Biol. Bull. (Woods Hole, Mass.)*, **124**, 31-44.

Goodbody, I., 1974. The physiology of ascidians. *Adv. mar. Biol.*, **12**, 1-149.

Gotelli, N.J., 1987. Spatial and temporal patterns of reproduction, larval settlement, and recruitment of the compound ascidian *Aplidium stellatum*. *Mar. Biol.*, **94**, 45-51.

Grace, J. B. and Wetzel, R. G., 1981. Habitat partitioning and competitive displacement in cattails (*Typha*): experimental field studies. *Am. Nat.*, **118**, 463-474.

Grave, C., 1920. *Amaroucium pelludidum* (Leidy) form *Constellatum* (Verrill). 1. The activities and reactions of the tadpole larvae. *J. exp. Zool.*, **30**, 239-259.

Grave, C., 1926. *Molgula citrina* (Alder and Hancock). Activities and structure of the free swimming larva. *J. Morphol.*, **42**, 453-471.

Grave, C., 1935. Metamorphosis of ascidian larvae. *Pap. Tortugas. Lab.*, **29**, 211-291.

Grave, C., 1941. The eye spot and light responses of the larvae of *Cynthia partita*. *Biol. Bull. mar. biol. Lab., Woods Hole.*, **81**, 287.

Grave, C., 1944. The larva of *Styela (Cynthia) partita:* structure, activities and duration of life. *J. Morphol.*, **75**, 173-191.

Grave, C. & Woodbridge, H., 1924. *Botryllus schlosseri* (Pallas): The behaviour and morphology of the free-swimniing larva. *J. Morph.*, **39**, 207-247.

Grosberg, R.K., 1982. Intertidal zonation of barnacles: the influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology*, **63**, 894-899.

Guiry, G.M. & Guiry, M.D., 1973. Spread of an introduced ascidian to Ireland. *Mar. Poll. Bull.*, **4**, 127.

Gulliksen, B., 1972. Spawning, larval settlement, growth, biomass, and distribution of *Ciona intestinalis* L. (Tunicata) in Borgenfjorden, North-Tröndelag, Norway. *Sarsia*, **51**, 83-96.

Gulliksen, B. & Skjæveland, S.H., 1973. The sea-star, *Asterias rubbens* L., as predator on the ascidian, *Ciona intestinalis* (L.), in Borgenfjorden, North-Tröndelag, Norway. *Sarsia*, **52**, 15-20.

Harant, H. & Verières, P., 1933. Faune de France. 27. Tuniciers. Fascicule 1: Ascidies. Lechevalier, Paris. 99p.

Hardin, G., 1960. The competitive exclusion principle. *Science*, **131**, 1292-1297.

Hastings, A., 1990. Spatial heterogeneity and ecological models. *Ecology*, **71**, 426-428.

Hills, J.M. & Thomason, J.C., 1996. The effect of scales of surface roughness on the settlement of barnacle cyprids (*Semibalanus balanoides*). Paper presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966.

Hirai, E. & Tsubata, B., 1956. In: West, A.B. & Lambert, C.C., 1976. Control of spawning in the tunicate *Styela plicata* by variations in a natural light regime. *J. Exp. Zool.*, **195**, 263-270.

Hobson, E. S. & Chess, J.R., 1978. Trophic relationships among fishes and plankton in the lagoon at Enewetak Atoll, Marshall Islands. *Fish. Bull.*, **76**, 133-153.

Holloran, M.K. & Witteman, G.J., 1986. Diurnal periodicity in planula release by the reef coral *Pocillopora damicornis*. In: *Coral Reef Population Biology*, edited by P. L. Jokiel, R. R. Richmond and R. A. Rogers. Hawaii Institute of Marine Biology Technical Report No.37. pp. 161-166.

Holmes, N.J., 1968. Aspects of the biology of *Styela clava* Herdman. PhD thesis, University of Southampton. 176p.

Holmes, N.J., 1971. The ascidian fauna of Southainpton Water. CERL Laboratory Note No. RD/L/N 187/71. Central Electricity Research Laboratories, Leatherhead.

Holmes, N.J., 1972. Water transport rates of *Styela clava* in fluctuating temperatures. CERL Laboratory Note No. RD/L/N 27/72. Central Electricity Research Laboratories, Leatherhead.

Holmes, N.J. & Coughlan, J., 1975. The ascidian fauna of Southainpton Water. *Proc. Hants. Field Club Archaeol. Soc.*, **30**, 9-15.

Houghton, D.R. & Miller, R.H., 1960. Spread of the ascidian *Styela mammiculata* Carlisle. *Nature, Lond.*, **185**, 862.

Hurlbut, C.J., 1991a. The effects of larval abundance, settlement and juvenile mortality on the depth distribution of a colonial ascidian. *J. Exp. Mar. Biol. Ecol.*, **150**, 183-202.

Hurlbut, C.J., 1991b. Larval substratum selection and postsettlement mortality as determinants of the distribution of two bryozoans. *J. Exp. Mar. Biol. Ecol.*, **147**, 103-119.

Hutchinson, G.E., 1961. The paradox of the plankton. *Am. Nat.*, **93**, 137-145.

Huwae, P.H.M., 1974. *Styela clava* Herdman, 1882, (Tunicata Ascidiacea) nieuw voor Nederland. *Zeepaard*, **34**, 28. (in Dutch).

Huwae, P.H.M. & Lavaleye, M.S.S., 1975. *Styela clava* Herdman, 1882, (Tunicata Ascidiacea) nieuw voor Nederland. *Zool. Bijdr.*, **17**, 79-81. (in Dutch).

Jackson, J.B.C., 1979. Overgrowth competition between encrusting chieilostome ectoprocts in a Jamaican cryptic reef enviromnent. *J. Anim. Ecol.*, **48**, 805-823.

Jacoby, C.A., 1982. Behavioral responses of the larvae of *Cancer magister* Dana (1852) to light, pressure, and gravity. *Mar. Behav. Physiol.*, **8**, 267-283.

Jefferies, M.J. and Lawton, J.H., 1985. Enemy-free space and the structure of ecological communities. *Bio. J. Linn. Soc.*, **23**, 269-286.

Jeffery, W.R., 1990. Ultraviolet irradiation during ooplasmic segregation prevents gastrulation, sensory cell induction, and axis formation in the ascidian embryo. *Developmental Biology*, **140**, 388-400.

Jeffery, W.R., 1994. A model for ascidian development and developmental modifications during evolution. *J. mar. biol. Ass. U.K.*, **74**, 35-48.

Jerlov, N.G., 1970. General aspects of underwater daylight and definitions of fundamental concepts. In: *Marine Ecology. Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters, 1. Part 1*, edited by E. Kinne. John Wiley, New York. pp. 95-102.

Jokiel, P., 1980. Solar ultraviolet radiation and coral reef epifauna. *Sci.*, **207**, 1069-71.

Jørgensen, C.B., 1949. Feeding rates of sponges, lamellibranchs and ascidians. *Nature* (Lond.), **163**, 912.

Jørgensen, C.B., 1955. Quantitative aspects of filter feeding in invertebrates. *Biol. Rev.*, **30**, 391-454.

Jørgensen, C.B. & Goldberg, E.D., 1953, Particle filtration in some ascidians and lamellibranchs.. *Biol. Bull.*, **105**, 477.

Kajiwara, S. & Yoshida, M., 1985. Changes in behavior and ocellar structure during the larval life of solitary ascidians. *Biol. Bull.*, **169**, 565-577.

Kampa, E.M., 1970. Photoenvironment and sonic scattering. In: Farquhar, G.B. (ed.) *Proceedings of an international symposium on biological sound scattering in the ocean.* Maury Center for ocean science, Department of the Navy, Washington, D.C., pp. 51-59.

Kang, P.A., Kim, Y. & Yoon, D.S., 1980. Studies on the hanging culture of oyster, *Crassostrea gigas*, in the Korean coastal waters. 4. On the fouling organisms associated with culturing oysters at the oyster culture farms in Chungmu. *Bull. Fish. Res. Dev. Agency, Busan*, no. 25, 29-34. (In Korean).

Katz, M.J., 1983. Comparative anatomy of the tunicate tadpole, *Ciona intestinalis*. *Biol. Bull.*, **164**, 1-27.

Kelly, D.L., 1974. Aspects of the reproductive ecology of three solitary ascidians, *Ciona intestinalis* (L.), *Styela plicata* (L.), and *Styela clava* (H.), from Southern California. MA thesis. California State University, Fullerton. 51p.

Keough, M.J., 1988. Benthic populations: is recruitment limiting or just fashionable? In: *Proceedings of the Sixth International Coral Reef Symposium, Australia, 1988, Vol. 1*, edited by J.H.Choat *et al.*, Sixth International Coral Reef Symposium Committee, Townsville, Australia. pp. 141-148.

Keough, M.J. & Downes, B.J., 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. *Oecologia (Berlin)*, **54**, 348-352.

Knaben, N., 1952. Development of the larvae of *Ascidiella aspersa* (Müll.) at different salinities and temperatures. *Avh. utg. av Det Norske Videnskaps-Akademi i Oslo, I., Mat.-Naturv. Klasse*. No.3. 1-24.

Knight-Jones, E.W. & Morgan, E., 1966. Responses of marine animals to changes in hydrostatic pressure. *Oceanogr. Mar. Biol. Ann. Rev.*, **4**, 267-299.

Knight-Jones, E.W. & Qasim, S.Z., 1955. Responses of some marine plankton animalsto changes in hydrostatic pressure. *Nature*, **175**, 941-942.

Knight-Jones, E.W. & Qasim, S.Z., 1966. Response of crustacea to changes in hydrostatic pressure. In: Proceedings of the Symposium on Crustacea. Mar. biol. Ass. India, Part III, 1132-1150.

Lack, D., 1947. *Darwin's Finches*. Cambridge University Press. 208 pp.

Lambert, C.C. & Brandt C.L., 1967. The effect of light on the spawning of *Ciona intestinalis*. *Biol. Bull. (Woods Hole, Mass.)*, **132**, 222-228.

Lambert, C.C. & Lambert, G., 1979. Tunicate eggs utilise ammonium ions for flotation. *Science*, **200**, 64-65.

Lambert, G., 1968. The general ecology and growth of a solitary ascidian, *Corella willmeriana*. *Biol. Bull. (Woods Hole, Mass.)*, **135**, 296-307.

Lane, D.J.W., 1973. Attachment of the larva of the ascidian *Diplosoma listerianum*. *Mar. Biol.*, **21**, 47-58.

Latz, M.I. & Forward, R.B., Jr., 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biol. Bull. (Woods Hole, Mass.)*, **153**, 163-179.

Lawton, J.H. and Hassell, M.P., 1981. Asymmetrical competition in insects. *Nature*, **289**, 793-795.

Levins, R., 1968. Evolution in changing environments. Some theoretical explorations. *Monographs in Population Biology*, 2. Princetown University Press.

Lindsay, S.T. & Thompson, H., 1930. The determination of specific characters for the identification of certain ascidians. *J. mar. biol. Ass. U.K.*, **17**, 1-45.

Lützen, J. & Sørensen V., 1993. Ecology, reproduction and further spread of the immigrant East-Asiatic ascidian *Styela clava* Herdman in Danish waters. *Flora og Fauna*, **99**, 75-79. (In Danish).

MacArthur, R.H., 1955. Fluctuations of animal populations and a measure of community stability. *Ecology*, **36**, 533-536.

MacArthur, R.H. & Levins, R., 1964. Competition, habitat selection and character displacement in a patchy environment. *Proc. Nat. Acad. Sci.*, **51**, 1207-1210.

Mast, S.O., 1921. Reactions to light in the larvae of the ascidians, *Amaroucium constellatum and Amaroucium pellucidum* with special reference to photic orientation. *J. Exp. Zool.*, **34**, 149-87.

Meadows, P.S. & Campbell, J.I., 1972. Habitat selection by aquatic invertebrates. *Adv. mar. Biol.*, **10**, 271-382.

M.H.L.G., 1967. Wessex Rivers: Hydrological Survey. Ministry of Housing and Local Government. H.M.S.O.

Millar, R.H., 1952. The annual growth and reproductive cycle in four ascidians. *J. mar. biol. Ass. U.K.*, **31**, 41-61.

Millar, R.H., 1953. Ciona. L.M.B.C. Memoirs of typical British marine plants and animals. **XXXV**. The University Press of Liverpool. 123p.

Millar, R.H., 1960. The identity of the ascidians *Styela mammiculata* Carlisle and *S. clava* Herdman. *J. mar. biol. Ass. U.K.*, **39**, 509-511.

Millar, R.H., 1969. Catalogue of main marine fouling organisms. Vol.4. Ascidians of European Waters. O.E.C.D. Publications, Paris. 34p.

Millar, R.H., 1970. British Ascidians. Synopses of the British Fauna No. 1. The Linnean Society of London. Academic Press. 92p.

Millar, R.H., 1971. The biology of ascidians. *Adv. mar. Biol.*, **9**, 1-100.

Miller, S.E. & Hadfield, M.G., 1986. Ontogeny of phototaxis and metamorphic competence in larvae of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opistobranchia). *J. Exp. Mar. Biol. Ecol.*, **97**, 95-112.

Mills, K.J.A., 1984. A study of the Ascidiacea from Southampton Docks. M.Sc. thesis, University of Southampton. 96p.

Minchin, D. & Duggan, C.B., 1988. The distribution of the exotic ascidian, *Styela clava* Herdman, in Cork Harbour. *Ir. Nat. J.*, **22**, 388-393.

Minganti, A., 1957. Inhibition of melanogenesis in *Phallusia* embryos (ascidians). *Acta Embryol. Morphol. Exptl.*, **1**, 37-47.

Monniot, C., 1970. Sur quatre ascidies rares ou mal connues des côtes de la Manche. *Cah. Biol. Mar.*, **11**, 145-152. (In French).

Moore, R.M., 1978. *Trace metals, dissolved organic matter and their association in natural waters.* Ph.D. thesis, University of Southampton.

Morgan, E., 1984. The pressure responses of marine invertebrates: a psychophysical perspective. *Zool. J. Linn. Soc.*, **80**, 209-230.

Morgan, T.H., 1945. The conditions that lead to normal or abnormal development of *Ciona*. *Biol. Bull.*, **88**, 50-62.

Na, G.H. & Lee, T.Y., 1977. Early development and larval distribution of ascidians *Styela clava* Herdman and *Ciona intestinalis* (Linne). *Publ. Inst. Mar. Sci. Natl. Fish. Univ. Busan.*, **10**, 41-56. (In Korean).

Nelson, T.C., 1928. Pelagic dissoconchs of the common mussel, *Mytilus edulis*, with observations on the behaviour of the larvae of allied genera. *Biol. Bull.*, **55**, 180-192.

Nicholson, A. J., 1954. An outline of the dynamics of animal populations. *Aust. J. Zool.*, **2**, 9-65.

Olson, R.R., 1983. Ascidian-*Prochloron* symbiosis: the role of larval photoadaptations in midday larval release and settlement. *Biol. Bull. (Woods Hole, Mass.)*, **165**, 221-240.

Olson, R.R., 1985. The consequences of short distance larval dispersal in a sessile marine invertebrate. *Ecol.*, **66**, 30-39.

Ott, F.S. & Forward, R.B., Jr., 1976. The effect of temperature on phototaxis and geotaxis by larvae of the crab *Rhithropanopeus harrisi* (Gould). *J. Exp. Mar. Biol. Ecol.*, **23**, 97-107.

Park, T. 1954. Experimental studies of experimental competition 11. Temperature, humidity and competition in two species of *Tribolium*. *Physiological Zoology*, **27**, 177-238.

Phillips, A.J., 1980. Distribution of chemical species. In: *The Solent Estuarine System - an assessment of present knowledge*. NERC Publications Series C, No.22, 44-61.

Pires, A. & Woollacott, R.M., 1983. A direct and active influence of gravity on the behaviour of a marine invertebrate larva. *Science*, **220**, 731-3.

Qian, P.-Y. & Bryan, P.J., 1996. Induction of settlement and metamorphosis in the abalone *Haliotis diversicolor*. Paper presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966.

Randløv, A. & Riisgård, H.U., 1979. Efficiency of particle retention and filtration rate in four species of ascidians. *Mar. Ecol. Prog. Ser.*, **1**, 55-59.

Reese, J.P., 1967. In: West, A.B. & Lambert, C.C., 1976. Control of spawning in the tunicate *Styela plicata* by variations in a natural light regime. *J. Exp. Zool.*, **195**, 263-270.

Rice, A.L., 1964. Observations on the effects of changes of hydrostatic pressure on the behaviour of some marine animals. *J. mar. biol. Ass. U.K.*, **44**, 163-175.

Rice, A.L., 1966. The orientation of pressure responses of some marine Crustacea. In: Proceedings of the Symposium on Crustacea. Mar. biol. Ass. India, Part III, 1124-1131.

Richmond, R.H. & Jokiel, P.L., 1984. Lunar periodicity in larval release in the reef coral *Pocillopora damicornis* at Enewetak and Hawaii. *Bull. Mar. Sci.*, **34**, 280-287.

Roberts, D., Rittschof, D., Holm, E. & Schmidt, A.R., 1991. Factors influencing initial larval settlement: temporal, spatial and surface molecular components. *J. Exp. Mar. Biol. Ecol.*, **150**, 203-211.

Robinson, W.E., Kusten, K. & Cloney, R.A., 1986. The influence of tunichrome and other reducing compounds on tunic and fin formation in embryonic *Ascidia callosa* Stimpson. *J. Exp. Zool.*, **237**, 63-72.

Rose, S.M., 1939. Embryonic induction in the Ascidia. *Biol. Bull. (Woods Hole, Mass.)*, **77**, 216-232.

Ross, H.H., 1957. Principles of natural coexistence indicated by leafhopper populations. *Evolution*, **11**, 113-129.

Ryland, J.S., 1960. Experiments on the influence of light on the behaviour of polyzoan larvae. *J. exp. Biol.*, **37**, 783-800.

Sabbadin, A., 1957. Il ciclo biologico di *Ciona intestinalis* (L.), *Mogula manhattensis* (DeKay), e *Styela plicata* (Lesueur), nella laguna Veneta. *Adch. Di Oceanografia e Limnologia*, **Vio XI**, 1-29. (in Italian).

Satuito, C.G., Shimizu, K., Natoyama, K., Yamazaki, M. & Fusetani, N., 1996. Factors influencing attachment and metamorphosis of larvae of the mussel *Mytilus edulis galloprovincialis*. Paper presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966

Schembri, P.J., 1982. Locomotion, feeding, grooming and the behavioural responses to gravity, light and hydrostatic pressure in the stage 1 zoea larvae of *Ebalia tuberosa* (Crustacea: Decapoda: Leucosiidae). *Mar. Biol.*, **72**, 125-134.

Schmidt, G.H., 1982. Aggregation and fusion between conspecifics of a solitary ascidian. *Biol. Bull. mar. biol. Lab., Woods Hole*, **162**, 195-201.

Schmidt, G.H. & Warner, G.F,, 1984. Effects of caging on the development of a sessile epifaunal community. *Mar. Ecol. Prog. Ser.*, **15**, 251-263.

Schöne, H., 1975. Orientation in space: animals. General Introduction. In: Kinne, 0. (ed.) *Marine Ecology, Vol. 11. Physiological Mechanisms*. Wiley, London, pp. 499-553.

Shorrocks, B., Rosewell, J., Edwards, K. and Atkinson, W., 1984. Interspecific competition is not a major organising force in many insect communities. *Nature*, **310**, 310-312.

Smith, R.C. & Baker, K.S., 1979. Penetration of UV-B and biologically effective dose-rates in natural waters. *Photochem. Photobiol.*, **29**, 311-323.

Spooner, G.M., 1933. Observations on the reactions of marine plankton to light. *J. mar. biol. Ass. U.K.*, **19**,385-438.

Stoner, D.S., 1990. Recruitment of a tropical colonial ascidian: relative importance of pre-settlement vs. post-settlement processes. *Ecology*, **71**, 1682-1690.

Strong, D.R., Lawton, J.H. and Southwood, T.R.E., 1984. Insects on plants: Community Patterns and Mechanisms. Blackwell Scietific Pub., Oxford. 313 pp.

Sulkin, S.D., 1975. The influence of light in the depth regulation of crab larvae. *Biol. Bull. (Woods Hole, Mass.)*, **148**, 333-343.

Sulkin, S.D., 1984. Behavioral basis of depth regulation in the larvae of brachyuran crabs. *Mar. Ecol. Prog. Ser.*, **15**, 181-205.

Sulkin S.D., van Heukelem, W., Kelly P. & van Heukelem L., 1980. The behavioral basis of larval recruitment in the crab *Callinectes sapidus* Rathbun: a laboratory investigation of ontogenetic changes in geotaxis and barokinesis. *Biol. Bull. (Woods Hole, Mass.)*, **159**, 402-417.

Sutherland, J.P. & Karlson R.H., 1977. Development and stability of the fouling community at Beaufort, North Carolina. *Ecol. Monogr.*, **47**, 425-446.

Svane, I., 1982. Possible ascidian counterpart to the vertebrate saccus vasculosus with reference to *Pyura tessellata* (Forbes) and *Boltenia echinata* (L.).*Acta Zool. (Stockholm)*, **63**, 85-89.

Svane, I., 1983. Ascidian reproductive patterns related to long-term population dynamics. *Sarsia*, **68**, 249-255.

Svane, I., 1987. On larval behaviour and post-metamorphic mortality of *Ascidia mentula* O.F. Müller. *Ophelia*, **27**, 87-100.

Svane, I. & Havenhand J.N., 1989. Scale of dispersal in *Ciona intestinalis* L.: is planktonic development and dispersal facultative? Poster presented at *Trophic relationships in the marine environment*, the 24th European Marine Biology Symposium., Oban 4-10 Oct. 1989.

Svane & Havenhand 1993

Svane, I. & Young C.M., 1989. The ecology and behaviour of ascidian larvae. *Oceanogr. Mar. Biol. Annu. Rev.*, **27**,45-90.

Tansley, A.G., 1917. On competition between *Gallium syvestre* L. (*G. hercynicum* Weig.) and *Gallium syvestre* Poll. (*G. asperum* Schreb.) on different types of soil. *J. EcoL*, **5**, 173-179.

Thorson, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Medd. Komm. Danm. Fisk. -og Havunders.*, ser. Plankton, **4**, 523 pp.

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, **25**, 1-45.

Thorson, G., 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia*, **1**, 167-208.

Timko, P., 1979. Larviphagy and oophagy in benthic invertebrates: a demonstration for *Dendraster excentricus* (Echinoidea). In: *Reproductive Ecology of Marine Invertebrates*, ed. By S. Stancyk. University of South Carolina Press, South Carolina. p. 91-98.

Torrence, S.A. & Clony, R.A., 1982. Nervous system of ascidian larvae: caudal primary sensor neurons. *Zoomorphology*, **99**, 103 -115.

Tsurumi, K., Matsumura, K. & Fusetani, N., 1996. Effects of preliminary microbial films on settlement and metamorphosis of cypris larvae of the barnacle, *Balanus amphitrite* Darwin. Paper presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966

Underwood, A.J. & Denley, E.J., 1984. Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: *Ecological Communities: Conceptual Issues and the Evidence*, edited by D. Strong, D. Simberloff, L. G. Abele and A. B. Thistle. Princeton University Press. pp. 151-180.

Vogel, S.,1981. *Life in Moving Fluids. The physical Biology of Flow*. William Grant Press, Boston. 352 pp.

Waddington, C.H., 1957. *The Strategy of the Genes*. Allen and Unwin."

Wallace, H., 1961. The breeding and development of *Styela mammiculata* Carlisle. *J. mar. biol. Ass. U.K.*, **41**, 187-190.

Walne, P.R., 1961. Observations on the mortality of *Ostrea edulis*. *J. mar. biol. Ass. U.K.*, **41**, 113-122.

Walters, L.J., Miron, G. & Bourget, E., 1996. Direct observations of invertebrate larval exploration of substrata and settlement via endoscopy. Paper presented at the symposium *Settlement and Metamorphosis of Marine Invertebrate Larvae*. University of Plymouth, 15-18 July, 1966.

Webber, N.B., 1980. Hydrography and water circulation in the Solent. In: *The Solent Estuarine System - an assessment of present knowledge*. NERC Publications Series C, No.22, 25-35.

West, A.B. & Lambert, C.C., 1976. Control of spawning in the tunicate *Styela plicata* by variations in a natural light regime. *J. Exp. Zool.*, **195**, 263-270.

Westerweel, H., 1975. *Styela clava* Herdman, 1882, nu ook in Zeeland. *Zeepaard*, **35**, 99.

Westwood, I.J., 1980. Mixing and dispersion in Southampton Water. Ph.D. thesis, University of Southampton.

Whittaker, J.R., 1966. An analysis of melanogenesis in differentiating pigment cells of ascidian embryos. *Dev. Biol.*, **14**, 1-39.

Whittingham, D.G., 1967. Light-induction of shedding of gametes in *Ciona intestinalis* and *Molgula manhattensis*. *Biol. Bull. (Woods Hole, Mass.)*, **132**, 292-298.

Wilson, D.P.,1937. The influence of the substratum on the metamorphosis of *Notomastus* larvae. *J. mar. biol. Assoc. U.K.*, **22**, 227-243.

Wilson, D.P.,1948. The relation of the substratum to the metamorphosis of *Ophelia* larvae. *J. mar. biol. Assoc. U.K.*, **27**, 723-760.

Wilson, D.P.,1953. The settlement of *Ophelia bicornis* Savigny larvae. The 1951 experiments. *J. mar. biol. Assoc. U.K.*, **31**, 413-438.

Wilson, D.P.,1954. The attractive factor in the settlement of *Ophelia bicornis* Savigny. *J. mar. biol. Assoc. U.K.*, **33**, 361-380.

Woodbridge, H., 1924. *Botryllus schlosseri* (Pallas). The behaviour of the larvae with special reference to habitat. *Biol. Bull. (Woods Hole, Mass.)*, **47**, 223-230.

Yamaguchi, M., 1970. Spawning periodicity and settling time in ascidians, *Ciona intestinalis and Styela plicata*. *Rec. oceanogr. Wks Japan*, **10**, 147-155.

Yamaguchi, M., 1975. Growth and reproductive cycles of the marine fouling ascidians *Ciona intestinalis, Styela plicata, Botrylloides violaceus*, and *Leptoclinum mitsukurii* at Aburatsubo-Moroiso inlet (Central Japan). *Mar. Biol.*, **29**, 253-259.

Yoshioka, P.M., 1982. Role of planktonic and benthic factors in the population dynamics of the bryozoan *Membranipora membranacea*. *Ecol.*, **63**, 457-468.

Yoshioka, P.M., 1986. Chaos and recruitment in the bryozoan *Membranipora membranacea*. *Bull. Mar. Sci.*, **39**, 408-417.

Young, C.M., 1982. Larval behavior, predation and early post-settling mortality as determinants of spatial distribution in subtidal solitary ascidians of the San Juan Islands, Washington. Ph.D. dissertation, University of Alberta. 260p.

Young, C.M., 1988. Ascidian cannibalism correlates with larval behavior and adult distribution. *J. Exp. Mar. Biol. Ecol.*, **117**, 9-26.

Young, C.M. & Braithwaite L.F., 1980. Larval behavior and post-settling morphology in the ascidian *Chelyosoma productum* Stimpson. *J. Exp. Mar. Biol. Ecol.*, **42**, 157-169.

Young, C.M. & Chia, F.-S., 1982. Ontogeny of phototaxis during larval development of the sedentary polychaete, *Serpula vermicularis* (L.). *Biol. Bull. (Woods Hole, Mass.)*, **162**, 457-468.

Young, C.M. & Chia, F.-S., 1984. Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. *Mar. Biol.*, **81**, 61-68.

Young, C.M. & Chia, F.-S., 1985. An experimental test of shadow response function in ascidian tadpoles. *J. Exp. Mar. Biol. Ecol.*, **85**, 165-175.

Young, C.M. & Chia, F.-S., 1987. Abundance and distribution of pelagic larvae as influenced by predation, behaviour, and hydrographic factors. In: *Reproduction of Marine Invertebrates, Vol. 9: General Aspects: Seeking Unity in Diversity*, edited by A. C. Giese *et al.*, Blackwell/Boxwood, Palo Alto and Pacific Grove, California, pp. 385-463.

Young, C.M. & Gotelli, N.J., 1988. Larval predation by barnacles: effects on patch colonisation in a shallow tidal community. *Ecology*, **69**, 624-634.

Zar, J.H., 1984. Biostatistical Analysis. (Second edition). Prentice-Hall International.